**ORIGINAL SUBMISSION**

NSF International

GRN 000582



May 19, 2015

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-255)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Attention: Dr. Paulette Gaynor

RE: GRAS Notification – Premium Agave Inulin - Resubmission

Dear Dr. Gaynor:

On behalf of IIDEA of Tlaquepaque Jalisco, México, we are resubmitting for FDA review a GRAS notification (original submission Nov. 11, 2011) for Premium Agave Inulin, trade name, Inufib™, a soluble dietary fiber which is to serve as a bulking agent or source of reduced energy carbohydrate. Please find enclosed one hard copy and one virus-free electronic copy (on CD). Based on your correspondence of May 29, 2012, and telephone correspondence with Lillian Shepherd of December 14, 2012, we understand that the Office of Food Additive Safety is seeking clarification on the intended uses and estimated daily intake of the agave inulin, and clarification of the contact person for this notification.

To address these requests, the following changes were made to the enclosed resubmitted notification:

1. The notifier has removed "baby foods" and "meats" from the original food and beverage categories proposed for addition of agave inulin (see Attachment 16).

2. The estimated daily intake (EDI) tables in Attachment 17 have been replaced with Table A, B, and C, reflecting use and intake levels for the updated use categories. These updated EDIs are reduced compared with the EDIs in the original notification, as summarized below:

| U.S. Consumer Groups | Estimated daily intake of inulin from Inufib™ from all food categories combined^ (grams per person-day) | | | |
|---|---|---|---|---|
| | Original Notification | | Current Resubmission | |
| | Mean | 90th percentile | Mean | 90th percentile |
| Ages ≥ 2 years | 10.1 | 19.2 | 8.4 | 16.8 |
| Ages ≥ 1 year to < 2 years | 7.6 | 13.7 | 6.5 | 13.2 |
| Ages < 1 year | 2.3 | 3.7 | 1.1 | 2.3 |
| ^Summarized from Tables in Attachment 17 in the original notification and current resubmission. | | | | |

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3. The contact for the notifier (Section 1.2) is as follows:

J. Caroline English, Ph.D., DABT
Senior Principal Toxicologist
NSF International
Mailing address: 151 Stone Pine Ln, Menlo Park, CA 94025
Mobile: 585-615-0417
Email: jenglish@nsf.org

4. An updated literature search was performed on April 8, 2015 to identify any new articles pertaining to the safety of agave inulin published since the previous literature searches conducted on April 13 and October 3, 2011. All relevant information retrieved as a result of the updated search was added in summary form to the current resubmission. All of the new information identified support and extend the previous Generally Recognized as Safe determination. The following new articles cited in the resubmission are enclosed:

Allsopp, P., S. Possemiers, D. Campbell, I.S. Oyarzábal, C. Gill, and I. Rowland. An exploratory study into the putative prebiotic activity of fructans isolated from *Agave angustifolia* and the associated anticancer activity. 2013. Anaerobe 22:38-44.

Dávila-Céspedes, A., B.I. Juárez-Flores, J.M. Pinos-Rodríguez, J.R. Aguirre-Rivera, A.C. Oros-Ovalle, E.D. Loyola-Martínez, and H. Andrade-Zaldívar. Protective Effect of *Agave salmiana* Fructans in Azoxymethane-Induced Colon Cancer in Wistar Rats. 2014. Nat Prod Commun 9(10): 1503-6.

Gracia M.I., M.M. Tinoco, H.M. Rivera, B.F. Sanchez, P.G. Tapia, L.M. Altamirano, R.L. Romero, and O.L. García. 2013. Acute Toxicity and Genotoxic Evaluation of Metlin® and Metlos® (Organic Agave Fructans). *Food and Nutrition Sciences* 4:106-12.

Hijová, E., V. Szabadosova, J. Štofilová, and G. Hrčková. Chemopreventive and metabolic effects of inulin on colon cancer development. 2013. J Vet Sci 14(4): 387-393.

Holscher, H.D., J.L. Doligale, L.L. Bauer, V. Gourineni, C.L. Pelkman, G.C. Fahey, Jr., and K.S. Swanson. 2014. Gastrointestinal tolerance and utilization of agave inulin by healthy adults. Food Funct 5(6): 1142-9.

López-Velázquez G., L. Díaz-García, A. Anzo, M. Parra-Ortiz, B. Llamosas-Gallardo, A.A. Ortiz-Hernández, J.Mancilla-Ramírez, J.M. Cruz-Rubio, and P. Gutiérrez-Castrellón. 2013. Safety of a dual potential prebiotic system from Mexican agave "Metlin® and Metlos®", incorporated to an infant formula for term newborn babies: a randomized controlled trial. Rev Inv Clin 65(6):483-90.

Márquez-Aguirre, A.L., R.M. Camacho-Ruiz, M. Arriaga-Alba, E. Padilla-Camberos, M. R. Kirchmayr, J.L. Blasco, and M. González-Avila. Effects of Agave *tequilana* fructans with different degree of polymerization profiles on the body weight, blood lipids and count of fecal *Lactobacilli/Bifidobacteria* in obese mice. 2013. Food Funct. 4(8), 1237-44.

Rendón-Huerta, J.A., B. Juárez-Flores, J.M. Pinos-Rodríguez, J.R. Aguirre-Rivera, and R.E. Delgado-Portales. 2012. Effects of Different Sources of Fructans on Body Weight, Blood Metabolites, and Fecal Bacteria in Normal and Obese non-diabetic and Diabetic Rats. Plant Foods Hum Nutr 67(1): 64-70.

Should you have any questions or concerns regarding this resubmitted GRAS Notification, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner. If additional information or clarification is needed, please do not hesitate to contact me.

Sincerely,

(b) (6)

J. Caroline English, Ph.D., DABT
Senior Principal Toxicologist
NSF International
jenglish@nsf.org

Enclosures: GRAS Notification – Agave Inulin 052015 (one hard copy)
GRAS Notification – Agave Inulin 052015 (one virus-free electronic copy on CD)
New articles cited in current resubmission

# Agave Inulin

## GENERALLY RECOGNIZED AS SAFE (GRAS) NOTIFICATION

Prepared for
**IIDEA (Industrializadora Integral del Agave SA de CV)**
**Av. Periférico Sur 7750,**
**Tlaquepaque Jalisco, México**

Prepared by
**NSF International**
**Ann Arbor, MI**

**May 2015**


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# 1.0 GRAS EXEMPTION CLAIM

## 1.1 Claim of Exemption from the Requirment for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

IIDEA (Industrializadora Integral del Agave SA de CV) has determined that its agave inulin product, which meets the specifications described in Section 3.5, is Generally Recognized As Safe (GRAS) in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination was made in concert with an appropriately convened panel of experts who are qualified by scientific training and experience. The GRAS determination is based on scientific procedures as described in the following sections. The evaluation accurately reflects the conditions of the intended uses of this ingredient in foods.

## 1.2 Name and Address of Notifier

IIDEA (Industrializadora Integral del Agave SA de CV)
Av. Periférico Sur 7750, Tlaquepaque Jalisco, México
FDA registration number: 13439186334

Contact: J. Caroline English, Ph.D., DABT, NSF International
Address: 151 Stone Pine Lane, Menlo Park, CA 94025
Email: jenglish@nsf.org Mobile: 585.615.0417

As the notifier, IIDEA accepts responsibility for the GRAS determination that has been made for agave inulin as described in the subject notification; consequently, agave inulin meeting the conditions described herein is exempt from pre-market approval requirements for food ingredients.

## 1.3 Common Name and Identity of the Notified Substance

The substance that is the subject of this Generally Recognized as Safe (GRAS) notification is agave inulin; trade name, Inufib™. It is the trade name used by IIDEA, for the inulin-type fructans derived from the piñas[1] (stems, also known as cores, hearts, or pines) of the agave plant, ***Agave tequilana* Weber var. *azul***, commonly known as blue agave and weber's blue agave.

Fructans are naturally occurring oligo- and polysaccharides with fructose as the repeating unit. Fructans from the blue agave, commonly called agave inulin, consist of branched inulin-levan type fructans, composed of fructose units joined by β(2→1) and β(2→ 6) glycosidic linkages, and 1,6-fructofuranose branches, with either a terminal 6-linked glucose molecule or an internally linked glucose molecule. The mean number of fructose units (i.e., degree of polymerization) in agave inulin is 16, with a range of 3 to 60 fructose units. Further details regarding the Carbohydrate Composition and Degree of Polymerization can be found in Section 9.1.1 and the Molecular Structure and Chain Length Distribution of Agave Inulin can be found in Section 9.1.2.

[1] Stems, also known as cores, hearts, or pines.

### 1.4 Conditions of Intended Use

GRAS status is being sought for agave inulin in the form of solid and liquid Inufib™ products, for use in a variety of foods and beverages in which it serves as a bulking agent or source of reduced energy carbohydrate for uses as a sugar replacer, fat-replacer and/or texture modifier, at per serving levels that reflect good manufacturing practices principles in that the quantity added to foods should not exceed the amount reasonably required to accomplish its intended technical effect.

Agave inulin is a soluble dietary fiber which is obtained from the tequilana weber agave plant. It is a non-digestible, prebiotic food ingredient that selectively promotes the growth and/or activity of beneficial bacteria in the colon, specifically, bifidobacteria and lactobacilli (Lopez and Urias-Silvas, 2007). It is not digested in the upper gastrointestinal tract, resulting in reduced caloric value, and will not lead to a rise in serum glucose or stimulate insulin secretion (Urias-Silvas et al., 2008). Agave inulin has a neutral, sweet, clean flavor, and is used to improve the mouth feel, stability and acceptability of low fat foods. It can be used to fortify foods with fiber and to improve the flavor and sweetness of low calorie foods. It also improves the texture of fat-reduced foods. Agave inulin is highly soluble in cold water, and can easily be incorporated into beverages, bakery products, and dairy products. Agave inulin has a unique ability to add textural properties to food. Inulin gels are very creamy and fat-like, and as such can be used as bulking agents and in fat reduction and fat replacement. Agave inulin also serves as a source of reduced energy carbohydrate for use as a sugar replacer.

The per serving levels added to foods and beverages range from 2 – 8 g inulin.

### 1.5 Basis for GRAS Determination

Pursuant to 21 CFR § 170.30, agave inulin as Inufib™ was determined to be GRAS through scientific procedures. The basis for the GRAS determination is discussed more fully in Section 5.0.

### 1.6 Availability of Information

The data and information that serve as the basis for this GRAS notification will be sent to the U.S. Food and Drug Administration (FDA) upon request or are available for review and copying at reasonable times at the offices of NSF International located at 789 N. Dixboro Rd, Ann Arbor, MI, 48105.

## 2.0 INTRODUCTION

### 2.1 Objective

At the request of IIDEA through their agent MASO Consulting (Irvine, Texas), NSF International has undertaken an independent safety evaluation of IIDEA's inulin from premium agave preparation, Inufib™. The preparation is composed primarily of inulin-type fructans extracted by milling from the piñas of the agave plant, ***Agave tequilana*** **Weber var.** ***azul***, commonly known

as blue agave and Weber's blue agave. These fructans consist of naturally occurring fructose polysaccharides with a range of 3 to 60 fructose units and mean of 16. The preparation also contains minor amounts of monosaccharides (fructose and glucose) and disaccharide (sucrose). The purpose of the evaluation is to ascertain whether the intended food uses of Inufib™ as a general purpose bulking agent, texturizing agent, or source of reduced energy carbohydrate are generally recognized as safe, i.e., GRAS, under the intended conditions of use.

### 2.2 Foreword

IIDEA, through its agent MASO, provided NSF International with substantial background information needed to enable the GRAS assessment to be undertaken. In particular, the information provided addressed the chemical and molecular composition, safety/toxicity of agave inulin, history of use of in food; and specifications, and method of preparation of IIDEA's Inufib™**.** IIDEA was asked to provide adverse reports, as well as those that supported conclusions of safety, and was asked to supply past and present human food use information. Knowing how much inulin has been safely consumed, i.e., the so-called "doses" or use levels, is critical in extrapolating to safe exposures for inulin from premium agave when consumed as a food ingredient. The composite safety/toxicity studies, in concert with exposure information, ultimately provide the specific scientific foundation for the GRAS determination.

IIDEA supplied the requested documentation, which was augmented with independent searches of the scientific and regulatory literature extending through April 8, 2015**.** A GRAS assessment based primarily on the composite safety information, that is, based on scientific procedures, with supportive information provided by common use in food, was undertaken. Those references that were deemed pertinent to the objective at hand are listed in Section 8.0.

### 2.3 Summary of Regulatory History of Inulin

Inulin-type fructans are a form of nondigestable soluble fiber such as that found in oat, wheat, chicory, and agave. Inulin-type fructans are virtually unabsorbed from the gastrointestinal tract and not hydrolyzed by human digestive enzymes. They are associated with an increase in fecal weight.

Roberfroid et al. (1998) describe the commercial use of inulin-type fructans and oligofructose in the U.S., Japan and Europe, where they are added to foods for their nutritional properties and dietary fiber content (typically 3–6 g per portion). As a macronutrient substitute, inulin is used to replace fat (0.25 g inulin replaces 1 g of fat) such that inulin concentrations are 2–6 g per portion. Likewise oligofructose is used as a sugar substitute mainly in dairy products and bakery products, at typically 2–6 g per portion (Coussement 1999). Inulin-type fructans from various botanical sources have been sold under various brand names and incorporated in a wide variety of food and beverage products to replace fat and sugar. Fructans are also used as texturing agents, foam stabilizers, or for improved mouth feeling in miscellaneous food products. Consumption of inulin-type fructans incorporated into baked goods, dairy products, baby foods, infant formulas, meat products and a large variety of processed foods and beverages is commonplace, at least since 1992, at which time the fat replacing potential of inulin was discovered and patented by Orafti

(Roberfroid and Delzenne, 1998). In other applications, inulin or oligofructose are added to allow a specific nutritional claim regarding the bifidogenic activity (typically 3 – 8 g per portion).

In the United States, chicory inulin was determined to be GRAS without questions by FDA (FDA 2003, GRN 118) and fructooligosaccharide a shorter chain length fructan produced by enzymatic synthesis from sucrose), was determined to be GRAS without questions by FDA (FDA, 2000b GRN 44).

Inulin has been approved for use as an acceptable food or food ingredient in most countries including all EU countries, Australia, Canada, and Japan (Franck, 2002). As a food or food ingredient, inulin can be used without specific limitations as ingredients in foods and drinks. A specific AOAC method of analysis was developed for fructans (AOAC 997.08) to accurately measure the content of inulin and oligofructose (Coussement, 1999).

In March, 2006, Canada's Health Products and Food Branch approved the classification of inulin as a dietary fiber in Canada, a decision that will allow ORAFTI to label its Beneo™ inulin as a "dietary fiber." ORAFTI Active Food Ingredients is a worldwide market leader in production and marketing of chicory-based food ingredients Beneo™ inulin and oligofructose. The Canadian Food Inspection Agency (2011) lists "chicory root inulin" as a traditional fiber source with the following specifications required for labeling:

> Specifications for standard inulin from chicory root (dwb): Appearance: white powder; Total fiber: 90% up to >98% (AOAC 997.08 or AOAC 999.03 method); Sugars: 5-11%; Max 2% if desugared; Degree of polymerization (DP) range: 2-60 (2-44 for late harvest); Average DP: 7-14; Molecules with DP < 10: 30-36%, up to 59% for late harvest; Molecules with DP < 20: 63-71% (up to 88% for late harvest); Molecules with DP = 20: 29-37% (min 12% for late harvest).

"Inulin from Jerusalem artichoke tuber" is similarly classified as a traditional fiber source (Canadian Food Inspection Agency, 2011); no specifications are included.

Inulin is classified as an allowable food ingredient under the European Directive 95/002 on Food Additives (EC, 1995), and all the European Union (EU) countries list inulin as having food ingredient status.

Food manufacturers have added inulin-derived substances to the general food supply in Australia and New Zealand since the mid 1990s. The technological purpose for addition to food is to emulsify or thicken food, or for nutritional reasons, such as for their prebiotic effect or as dietary fiber. Since 2001, inulin has appeared in a wide range of foods and is predominantly labelled as dietary fiber (Food Standards Australia New Zealand, FSANZ, 2008).

# 3.0 CHEMISTRY AND MANUFACTURE OF AGAVE INULIN

## 3.1 Description of Agave and Agave Inulin

The agave genus includes about 275 species belonging to the Asparagalus order and Agavaceae family. Four major parts of the agave are edible: the flowers, the leaves, the stem or basal rosettes, and the sap, called *aguamiel* (Davidson, 1999), and have been used by indigenous peoples for food and beverage since pre-Columbian times (Slauson, 2001). Analysis of several species of agave plant have shown that nonstructural, water soluble carbohydrates known as fructosans are the major fraction and are concentrated in the stem (Srinivasan and Bathia, 1953; Srinivasan and Bathia, 1954). There are several commonly used foods and beverages that originate from the juice or sap of the agave piñas. Blue tequilana Webber (or Weber) agave, known as blue agave, is grown in the state of Jalisco and is best known for its juices, or aguamiel, which is the base for distilling tequila. Originally blue agave was selectively bred for its short maturation cycle, flavorful baking qualities and ease of processing.

The freshly extracted juice or sap is drunk as a beverage known as aguamiel (honey-water), and the fermented beverage from this juice is a nutrient-rich brew known as pulque; both are popular beverages in the south of the Sonoran Desert (Debnath et al. 2010). Pulque is used as a regular dietary item in the central highlands of Mexico; it is mildly alcoholic and is consumed especially during festivals and significant cultural events such as religious holidays and weddings. Pulque has been studied extensively for its nutritional potential among traditional and indigenous populations, and serves as an example of how local food-based strategies can be used to ensure micronutrient nutrition (Kuhnlein, 2004; Hackman et al. 2006).

The freshly extracted juice is also a source of inulin, which can be prepared as a concentrated liquid or as a dried powder for use as a food or food ingredient. Agave inulin is a naturally occurring fructose polysaccharide that belongs to a class of carbohydrates known as fructans, and is the principal carbohydrate that occurs naturally in *Agave tequilana* Weber var. *azul*. Fructans are oligomers or polymers consisting of a chain of β-fructofuranosyl units connected to the fructose residue of a sucrose molecule through β(2→1) and/or β(2→6) linkages. The degree of polymerization (DP) of fructans varies from 2 to 60; by convention the polysaccharides are called inulins when the DP is greater than 10 and referred to as fructooligosaccharides or oligofructose when the DP is ≤ 10 (Niness, 1999; Corradini et al., 2004; Ortiz-Basurto et al. 2008). Inulin can be produced with minimal processing of agave piñas. It has been promoted as a natural dietary source of soluble fiber for incorporation into breakfast cereals [http://www.foodprocessing.com/articles/2010/breakfast-cereal.html, accessed September 8, 2011].

Agave fructans are the starting material for other common food and beverage products of agave, made by further processing of the fructans. The Natural Standard Review for agave indicates that agave is a useful sugar alternative since fructans are 90% fructose and have a low glycemic index (Hackman et al. 2006). Cooking the piña or otherwise treating the fructan polysachharides to hydrolyze them into their component fructose monomers is a method of commercial fructose production, and can also be used to produce fructose-based syrups. Agave syrup was developed and regulated by Mexico in the 1990s. Agave syrups are made from at least half a dozen plant

varieties, the most popular being blue agave, *Agave salmiana, Agave americana* and *Agave mapisaga* (Debnath et al. 2010). The roasted agave piña is sweet and is sold in markets in Mexico in chunks to be eaten.

Juice of agave piñas is the starting material for distilled spirits. Mescal is made by steaming and mashing the piñas, allowing the juice to ferment with added liquid for several days, and distilling the resulting fluid. Several local varieties of mescal are made in Mexican villages within the agave habitat; the most well known variety is called Bacanora, named after the Sonoran town (Debnath et al. 2010). Tequila is perhaps the most well known of the distilled spirits derived from agave.

The food and beverage products from agave differ with respect to the species of agave used as raw material and the degree and types of processing steps used to produce the final product. Thus, *Agave vera-cruz* is grown as a commercial source of fructose; *Agave salmiana, A. potatorum,* and *A. angustifolia* are used in the production of mescal (Michel-Cuello et al., 2008; Pena-Alvarez et al., 2004); *Agave atrovirens, Agave americana,* and *Agave salmiana* are the sources of aguamiel and pulque; and only agave of the species *A. tequilana* Weber blue variety, grown near the town Tequila in Jalisco, can be used for tequila production. Foods and beverages derived from the agave piñas that are available in the United States include fructose and fructose-based syrups, inulin, and tequila.

## 3.2 Common or Usual Name

Inulin (synonym: inulina) or agave inulin (synonym: inulina de agave) are the common names of the fructans derived from the piñas of the agave plant. Other common names include blue agave inulin, fructans from agave, and inulin tequilana Weber blue agave.

Inulin from premium agave is the common or usual name of the inulin derived from piñas of ***Agave tequilana* Weber var. azul**, commonly known as blue agave and Weber's blue agave, grown and processed in the occidental region of Mexico, which is the subject of the GRAS evaluation. The specific substance that is the subject of this safety evaluation is identified as Inufib™ as produced and sold by IIDEA (Industrializadora Integral del Agave SA de CV, located at Av. Periférico Sur 7750, Tlaquepaque Jalisco, México). Trade names located for other agave inulin products are BioAgave™, Fructagave, Vivagave, Agavina and Predilife (Gomez et al. 2010) and Olifructine-SP.

## 3.3 Chemistry of Inufib™

### **3.3.1** Product description

Inufib™ is IIDEA's brand name for products containing inulin. Products are manufactured by the mechanical extraction of the juice from the pine (piñas) of the blue agave without the use of solvents or other chemicals.

The following description of inulin from premium agave, or Inufib™, is given on the IIDEA Company's website[2]

> Inulin is a prebiotic ingredient that belongs to a class of fibers known as fructans. A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon to improve host health.
>
> **Prebiotic Properties**
>
> • Resistance to digestion
> • Hydrolysis and fermentation by colonic microflora
> • Selective stimulation of growth of one or a limited number of bacteria in the feces
> • May repress the growth of pathogens for overall beneficial health
>
> The agave fructans are an important and emerging group of prebiotics. On an industrial scale, inulin is extracted from chicory, agave, or artichoke with physical treatments.

### **3.3.2** Product composition

The major components of Inufib™ are the fructan carbohydrates obtained by mechanical extraction of the juice from the stem of the Weber's blue agave plant. Inufib™ is available for use in two forms, dry and liquid. Liquid Inufib™ is the concentrated, filtered juice and contains approximately 80% inulin with up to ~20% mono- and disaccharides. Dry Inufib™ is the filtered juice concentrate that is spray dried to produce a white or yellowish white powder with a neutral odor. Dry Inufib™ contains approximately 90% inulin with up to ~10% mono- and disaccharides formulated as a powder. Details regarding the Carbohydrate Composition and Degree of Polymerization can be found in Section 9.1.1 and the Molecular Structure and Chain Length Distribution of Agave Inulin can be found in Section 9.1.2.

No processing aids or additives are included in the final Inufib™ products, and no proprietary or coloring ingredients are added. Based on gas chromatography-mass spectrometric analysis (method PT-USAI-FQ-EM-001) of IIDEA's purified agave inulin product, Inufib™, conducted by an external laboratory, concentrations of saponins and terpenes are below 0.1 ppm (see Attachment 1 "Saponins and Terpenes"). No saponins were detected, and under the conditions of analysis, the test laboratory concluded that, if the compounds ecogenin and ecogin were present in the sample, their concentrations would be < 7 ppb (see Attachment 2 "Letter saponins Ext Lab").

### **3.3.3** Food grade inulin identity specifications

The majority (>95%) of food-grade inulin available on the worldwide market is produced from chicory (Orafti, 2007). The Canadian Food Inspection Agency (2011) lists "chicory root inulin" as a traditional fiber source with the following specifications required for labeling:

> Specifications for standard inulin from chicory root (dwb): Appearance: white powder; Total fiber: 90% up to >98% (AOAC 997.08 or AOAC 999.03 method); Sugars: 5-11%; Max 2% if desugared; Degree of polymerization (DP) range: 2-60 (2-44 for late harvest); Average DP: 7-14; Molecules

[2] http://www.iidea.com.mx/inulin.php

with DP < 10: 30-36%, up to 59% for late harvest; Molecules with DP < 20: 63-71% (up to 88% for late harvest); Molecules with DP = 20: 29-37% (min 12% for late harvest).

Under the USDA National Organics Program (NOP), inulin (CAS # 9005-80-5: synonym "inulin oligofructose enriched"), is listed on NOP §205.606 which lists the only nonorganic agricultural ingredients that are allowed to be used in organic products. These nonorganic ingredients may only be used when the organic form is not commercially available. Organically produced inulin may be used to replace the nonorganic ingredients allowed in NOP § 205.606. NOP certified organic inulin from agave is among the products registered under USDA's "606organic" web site of organic sources for agricultural ingredients listed on NOP § 205.606. (See http://606organic.com/results.php?product=Inulin-oligofructose%20enriched.)

### 3.4 Production Processes

Manufacturing processes and analytical methods for commercially available plant-derived inulins and oligofructose have been described in the scientific and patent literature; those related to agave-derived fructans are noted below, and IIDEA's premium agave inulin manufacturing process is also discussed. The production process for agave inulin shares some commonalities with production processes for agave-derived fructose and fermented products like tequila, with important distinctions that are discussed.

#### **3.4.1** Scientific and patent literature on *Agave tequilana* and other *Agave* species

The *A. tequilana* plant is used for the production of three main products that are ingested; the alcoholic beverage, tequila, the natural sugar substitute, agave syrup, as well as the subject of this notification, the natural inulin-type fructan, agave inulin. Tequila and agave syrup differ from agave inulin in an important respect, specifically; production of tequila and agave syrup both involve the hydrolysis of the fructans into their component fructose monomers. The hydrolysis step, accomplished by thermal, acid or enzymatic treatments, or some combination thereof, is not applied in the case of agave inulin production.

Several patents have been developed for applications of agave as a raw material which include the use of fructans from agave as a natural prebiotic with high natural fiber content; as a sweetener having improved nutritional properties; and as an additive in foodstuffs and cosmetic preparations. Fructose syrup from agave has proposed applications for an organic sports drink and a sugar replacement based on reduced calories and low glycemic index.

#### **3.4.2** Comparison of agave inulin to other commercially available inulins

Most commercially available inulin and oligofructose are extracted from chicory roots (*Cichorium intybus*). As with agave inulin, the degree of polymerization (DP) of chicory-derived inulin varies with source of the plant and time of harvest. Hot water diffusion is used to extract inulin from the chicory root, and the dried product has an average DP of 10-12 with a chain length distribution from 2 to 60 and 6-10% content of free sugars as sucrose, fructose, and glucose. Agave inulin, sourced from the plant stem and produced in a manner similar to inulin from chicory, has an average degree of polymerization of about 16 and distribution from 3 and 60. Thus fructans extracted from chicory roots and agave stems contain (as dried wt%) up to ~10% of combined

mono and disaccharides, mainly sucrose and fructose, and approximately 90% inulin (Niness, 1999; Murphy, 2001). The product Raftiline HP, commonly used in the food industry based on its fat mimetic properties, is manufactured by removing the shorter-chain oligomers and residual sugars from chicory-derived inulin, and has an average DP of 25, with a molecular distribution ranges from 11 to 60. Oligofructose is derived in the same way as inulin, with the addition of an enzymatic hydrolysis step after extraction, such that chain lengths range from 2 to 10, with an average DP of 4. The commercially available inulin from Sigma is derived from *Dahlia tubers*, and is standardized to have an average DP of 27-29 (Zuleta and Sambucetti, 2001).

Structurally, these plant-derived inulins consist mainly of β(2→1)fructosyl–fructose links with chicory inulin containing 1-2% β(2→ 6)fructosyl–fructose branches; Dahlia inulin having 4-5% β(2→6)fructosyl–fructose branches (Hariono et al., 2009), and agave inulin having approximately 24% β(2→6)fructosyl–fructose branches (Franck and de Leenheer, 2004). β(2→1) and β(2→ 6)fructosyl–fructose linkages cannot be hydrolysed by pancreatic or brush-border digestive enzymes. Therefore, these fructans reach the colon undigested, where they are fermented by Bifidobacterium spp. and other lactic acid-producing bacteria (Lopez et al. 2003; Munjal et al. 2009).

The properties of inulins from chicory and agave are presented in the table below, which was obtained from the Tierra Group website, located at: http://www.thetierragroup.net/products/agaveinulin/ Accessed September 3, 2011 – site no longer active.

## Native Agave Vs. Chicory Fructan Comparision

Properties and Technical Data

| Property | Native chicory inulin | Native agave fructan |
|---|---|---|
| Degree of polymerization avg. (DP) | 8 - 10 | 10 - 15 |
| Molecular weight | DP < 60 | DP < 40 |
| DP 3 – 4 | 5.00% | 9.30% |
| DP 5 -9 | 20.30% | 21.10% |
| ≤DP 10 | 25.30% | 30.40% |
| <DP 20 | 61.30% | 71.40% |
| >DP 20 | 32.80% | 28.60% |
| Structure | β-2,1- | β-2,1-<br>β-2,6- (high degree branching) |
| Solubility @ 20° C w/ clarity | 9g/100g | >40g/100g |
| Prebiotic fiber (d.m. basis) | ≥ 90% | ≥ 90% |
| Free sugars (d.m. basis) | ≤ 10% | ≤ 10% |
| Calories | 1.50 kcal/g | 1.50 kcal/g |
| Degree of hygroscopicity | High | Low to medium |
| Viscosity | Low up to 30% w/w | Low |
| Relative sweetness vs. sucrose | < 10% | 20 - 30% |
| Gel formation (particle gels) | 30g/100g | Does not form gels |

- Significantly better solubility in cold and hot water than chicory inulin, providing greater homogeneity, enhanced beverage applications.
- Agave fructan offers improved product sensory attributes, such as taste and tongue sensation, due to enhanced solubility
- Agave fructan has lower hygroscopicity than native chicory inulin reducing caking, providing greater product stability.
- Binds 3 water molecules per monosaccharide unit, allows water activity reduction of products to values as low as 0.8.
- Both fructan types have equal levels of prebiotic fiber and calories per 100 g.

### **3.4.3** Processing to provide IIDEA's premium agave inulin

Manufacturing processes and analytical methods used by IIDEA for the production of Inufib™ are similar to those used for the manufacture of chicory-derived inulin (Franck, 2002) and other inulin products on the market. Production of inulin from premium agave involves the mechanical

extraction of the juice from the pine (piñas) of the blue agave without the use of solvents or other chemicals. IIDEA is registered with the U.S. FDA pursuant to section 305 of the U.S. Pubic Health Security and Bioterrorism Preparedness and Response Act of 2002, and the FDA Registration no. is 13439186334 (see Certificate of Registration Attachment 3 "FDA-IIDEA 2010 – 2011"). The manufacturing process complies with the international GMP standard IS0 21 000. The production process has been assessed to identify any reasonable potential hazards associated with the process and critical control points established to prevent, eliminate, or reduce potential hazards to acceptable levels. Potential biological, chemical and physical hazards have been addressed by the Hazard Analysis Critical Control Point (HACCP) Plan (Attachment 4 "HACCP Plan – Inulin (2)"), which has been certified by Global Standards Certification (see Attachment 5 "HACCP Certificate") and Silliker Global Certification Services (see Attachment 6 "Silliker Audit Recognition").

Inufib™ is mechanically extracted from the pines ("piñas") of the agave plant. When the plants are harvested, the leaves and roots are cut off and left in the fields for soil enrichment. It is important to emphasize that the agave inulin Inufib™ is derived from the piñas and is not derived from the leaves of the agave, because sap or extracts from the leaves from some agave species have been noted to contain saponins and raphides of calcium oxalate, thereby rendering them inedible (see Appendix Table A-1).

The harvested piñas are transported by conveyor into a mill and a series of extractors for sieving and squeezing. The inulin juice falls into tubs while the resulting bagasse is separated and removed. The extracted inulin juice undergoes three filtration steps and the juice is then concentrated by evaporation. The filters retain foreign matter, such as small stones, insects, soil, and fiber, plastic and metal particles. Filters of 0.5 microns remove any microorganisms that are possibly present and can be expected to remove raphides of calcium oxalate, which are 30–500 μm in length (Salinas et al. 2001), if present. The filters used are manufactured with materials that are approved by the US FDA (See Attachment 7 "Ficha Tecnica Bolsas", "FDA Datos acerca de bolsas de FS" and "Betafine – filters cartridges"). After a final filtration step the resulting liquid product is bottled, or alternatively, the concentrated juice is spray dried to a final concentration of greater than 95% dry matter. Please refer to Attachment 4 "HACCP Plan – Inulin (2)" for the process flow charts and process steps for Dry and Liquid product, and dried and liquid product specifications.

Microbiological analysis of the dry product includes coliform, yeast, mold and Salmonella (see Attachment 8 "Certificate of Quality" 1, 2, 3 and 4 for the dried product and "Test Report – Microbiological). Microbiological analysis of the liquid product includes coliform, yeast, and mold (see Attachment 9 "Certificate of Quality" 1, 2 and 3 for the liquid product).

Analyses for heavy metals (arsenic, lead, mercury, and cadmium), dioxins, PCBs, and pesticides are also performed (see Attachment 10 "Analysis Status").

Inufib™ production has received the following certifications:

- Organic product certified by the United States Department of Agriculture (USDA)[3], ECOCERT, BCS ÖKO-GARANTIE GMBH, Japanese Agricultural Standards (JAS) and Naturland
- Kosher certified by The Badatz Igud Rabbonim KIR
- Halal certified by the Islamic Food and Nutrition Council of America

**3.4.4** Pesticides

No fungicides, slimicides or other biocides are used by IIDEA in the production of Inufib™.

**3.4.5** Processing aids and process chemicals

As a processing aid, IIDEA adds Perlite as a filter aid in the production of Inufib™. No other chemicals or processing aids are used.

## 3.5 Properties and Finished Product Specifications

**3.5.1** Physical chemical properties and product specifications

Product properties and specifications for dry and liquid Inufib™ are provided in Table 1. See Attachment 11 "Data sheet – powder inulin premium" and Attachment 4 "HACCP Plan – Inulin(2)."

A study of sensory attributes plus microbiological analyses of the powder Inufib™ at room temperature, 35 and 45 °C was performed. The data support a room temperature shelf life of 275 days for odor, flavor, and fluidity; however the product did not change with respect to appearance, color, rancidity, or microbiological contamination during the study period (see Attachment 12, "Shelf life External Analysis"). On the basis of the data and review by the HACCP program which compared the Inufib™ products to the shelf life of similar products, shelf lives of 3 years for the dry Inufib™ and 3 months for the liquid Inufib™ were assigned (see Attachment 13).

[3] Organic foods production act of 1990, at http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5060370&acct=nopgeninfo

**Table 1. Properties and Specifications for Dry and Liquid Inufib™**

| Properties/Specifications | Dry Product | Liquid Product |
|---|---|---|
| *Physical Chemical Properties* | | |
| Moisture: | 0.5 – 4.0% | 27 – 31% |
| Density: | 0.6 – 0.8 g/ml | 1.34 – 1.36 g/ml |
| Concentration | NA | 69º – 73º Brix |
| pH: | 4.0 – 6.0 (1%) | 4.0 – 6.0 |
| Color: | White powder | 300 – 1000 ICUMSA |
| Storage stability: | Stable, hygroscopic | Stable, hygroscopic |
| Taste: | Slightly sweet | Slightly sweet |
| Aroma: | Neutral | Not reported |
| *Product Specifications* | | |
| Ash content: | Max. 5.0 % | < 0.7 % |
| Dry matter | 98.0-100 % total carbohydrates | ≥ 98.0 % carbohydrates |
| Composition: | ≥ 88.0% inulin | ≥ 80.0% Inulin |
| | ≤ 10.0% fructose | ≤ 15.0% Fructose |
| | ≤ 3.5% glucose | ≤ 5.0% Glucose |
| | ≤ 2.0% disaccharides | ≤ 2.0% Disaccharides |
| *Aflatoxin and Microbiological Contaminants* | | |
| Mesofilic | Max. 2,500 UFC | ≤2,500 UFC/g |
| Coliform | Max. 10 UFC | ≤10 UFC/g |
| Yeast and molds | Max. 100 UFC | ≤100 UFC/g |
| *Shelf Life* | | |
| Shelf life from date of manufacture | 3 years [4] | 3 months[5] |
| NA = not applicable | | |

**3.5.2** Pesticide and heavy metal contaminants

Agave inulin powder from IIDEA is analyzed for heavy metals and an extensive list of pesticides, dioxins, and PCBs. Analytical reports listing the individual contaminants, their respective analytical methods, and the results of the analyses, are provided in the Attachment 14 “Pesticides Silliker” and “Analysis Eurofins”). The results of the analyses indicate that contaminants are not present at levels of concern.

[4] As stated in Section 6.1.5, HACCP Plan – Inulin. See also “Data Sheet – Powder Inulin Premium”; “Shelf Life Inulin”; and “Shelf Life External Analysis,” attached.
[5] As stated in section 6.2.5, HACCP Plan- Inulin

### 3.5.3 Compositional analysis of Inufib™

The results of compositional and microbiological analyses of four lots of dry Inufib™ powder are presented in Table 2.

Table 2. Compositional analysis of dry Inufib™ powder

| | Specification per HACPP March 2011 | (b) (4) | (b) (4) | (b) (4) | (b) (4) |
|---|---|---|---|---|---|
| Appearance | | Creamy white fine powder | Creamy white fine powder | Creamy white fine powder | Creamy white fine powder |
| Total carbohydrates (%) | Min 98.0% | 99.02% | 99.89 | 99.02 | 99.13 |
| Inulin (%) | ≥ 88.0 | 91.98 | 90.00 | 90.98 | 91.47 |
| Fructose (%) | ≤ 10.0 | 4.95 | 4.73 | 5.66 | 4.62 |
| Dextrose (i.e. glucose) (%) | ≤ 3.5 | 0.45 | 2.32 | 0.60 | 0.71 |
| Sucrose (glucose-fructose disachharide) (%0 | ≤ 2.0 disaccharides | 0.60 saccharose | 0.93 saccharose | 0.69 saccharose | 1.18 saccharose |
| Other carbohydrates (%) | Max 6.0 | 1.03 | 1.91 | 1.09 | 1.15 |
| Microbiological | | | | | |
| Mesofilic (total count) | Max. 2,500 | 10 | 10 | 260 | 13 |
| Coliform (UFC/g) | Max. 10 | <10 | <10 | <10 | <10 |
| Yeast (UFC/g) | Max. 100 | <10 | <10 | <10 | <10 |
| Mold (UFC/g) | Max. 100 | <10 | <10 | <10 | <10 |
| Salmonella (in 25 g) | | Absent | Absent | Absent | Absent |

Additional analyses of organic agave inulin by Eurofins Analytics showed total fat content to be <0.5% of the composition. Fatty acid composition showed that ~ 2/3 is saturated fatty acids and ~1/3 is monounsaturated fatty acids. The following components were all <0.05%: docosadienoic acid C22:2 (n-6) – ω6; polyunsaturated fatty acids; total trans-fatty acids; omega-3 fatty acids; and omega-6 fatty acids. Non quantifiable fatty acids were also <0.05%. Mineral analysis showed sodium content to be 0.0353 g/100 g. The complete analytical results are provided in the Attachment 14 – "Analysis Eurofins."

The results of compositional and microbiological analyses of three lots of liquid Inufib™ are presented in Table 3.

Table 3. Compositional analysis of Inufib™ liquid

| | Specification per HACPP March 2011 | (b) (4) | (b) (4) | (b) (4) |
|---|---|---|---|---|
| Appearance | | Light amber | Light amber | Light amber |
| Total carbohydrates (%) | Min 98.0% | 98.04% | 98.27 | 98.92 |
| Inulin (%) | ≥ 80.0 | 89.63 | 89.89 | 90.00 |
| Fructose (%) | ≤ 15.0 | 4.59 | 3.13 | 4.54 |
| Dextrose (i.e. glucose) (%) | ≤ 5.0 | 0.50 | 1.57 | 1.65 |
| Sucrose (glucose-fructose disachharide) (%0 | ≤ 2.0 disaccharides | 0.62 saccharose | 1.09 saccharose | 1.11 saccharose |
| Other carbohydrates (%) | No specification | 2.70 | 2.59 | 1.62 |
| Microbiological | | | | |
| Mesofilic (total count) | Max. 2,500 | 414 | 338 | 359 |
| Coliform (UFC/g) | Max. 10 | <10 | <10 | <10 |
| Yeast (UFC/g) | Max. 100 | <10 | <10 | <10 |
| Mold (UFC/g) | Max. 100 | <10 | <10 | <10 |

### 3.6 Inufib™ Analytical Methods

The content of agave inulin and other carbohydrates in Inufib™ are assayed according to the industrial standard "Official Norm NMX-FF-110-SCFI-2008" promulgated by the Government of Mexico (NMX-FF-110-SCFI-2008 Productos Alimenticios – Jarabe de Agave Explicaciones y Métodos de Prueba). Other assay methods used are NOM-092-SSA1-1994 for total count of mesophylic aerobic microorganisms; NOM-112-SSA1-1994 for coliform microorganisms; NOM-111-SSA1-1994 for yeasts and molds; NOM-114-SSA1-1994 for Salmonella; NOM-117-SSA1-1997 for heavy metals; and NMX-F-591-SCFI-2010 for foreign matter. The fatty acid composition is determined with method EN ISO 15304; EN ISO 5508; EN ISO 5509. Other assay methods employed in the production of Inufib™ are referenced in Attachment 15 "Laboratory analyses."

## 4.0 INTENDED FOOD USE AND DIETARY ESTIMATES

### 4.1 Intended Uses of Inufib™ in Food

Agave inulin is a prebiotic ingredient that belongs to a class of fibers known as fructans. Agave inulin is an organic dietary soluble fiber which is extracted from the *A. tequilana* Weber plant. A prebiotic is a non-digestible food ingredient that beneficially affects the body by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon to improve body health. Agave inulin is not digested in the upper gastrointestinal tract, resulting in reduced caloric value. Consumption will not lead to a rise in serum glucose or simulate insulin secretion. In addition, agave inulin aids to increase calcium and magnesium absorption. Agave inulin has a neutral, sweet clean flavor and is used to improve the mouth feel, stability and

acceptability of low fat foods. It can be used to fortify foods with fiber and to improve the flavor and sweetness of low calorie foods. Agave inulin also improves the texture of fat-reduced foods. Agave inulin is highly soluble in cold water and can easily be incorporated into beverages, bakery products, and dairy products. Agave inulin has a unique ability to add textural properties to food. Inulin gels are very creamy and fat-like, and as such can be used as a bulking agent and in fat reduction and fat replacement. Agave inulin also serves as a source of reduced energy carbohydrates for use as a sugar replacer (excerpted from Attachment 16 – "Intended Use").

IIDEA intends to market its inulin from premium agave for incorporation into the same food and beverage categories, with the exceptions of baby food and meats, as proposed in GRN 118 by Imperial Sensus for the use of the inulin product Frutafit®. The use levels vary by food category, and the average (mean) and maximum (90$^{th}$ percentile) use levels of inulin in any consumer group from these uses of Inufib™ were estimated to be 8.4 g/person-day and 16.8 g/person-day, respectively (Attachment 17 – Table C). The amounts of inulin from premium agave to be added to foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods as required by FDA regulation.

### 4.2 Estimated Daily Intake of Inufib™ from Proposed Uses

Roberfroid et al. (1998) describe the commercial use of inulin-type fructans and oligofructose in the U.S., Japan and Europe, where they are added to foods for their nutritional properties and dietary fiber content (typically 3–6 g per portion). As a macronutrient substitute, inulin is used to replace fat (0.25 g inulin replaces 1 g of fat) such that inulin concentrations are 2–6 g per portion. Likewise oligofructose is used as a sugar substitute mainly in dairy products and bakery products, at typically 2–6 g per portion (Coussement 1999)[6]. Fructans are also used as texturing agents, foam stabilizers, or for improved mouth feeling in miscellaneous food products (e.g., fermented dairy products; desserts such as jellies and ice creams; bakery products including biscuits, breads, and pastries; spreads; and infant formulas) (Roberfroid and Delzenne, 1998). In other applications, inulin or oligofructose is added to allow a specific nutritional claim regarding the bifidogenic activity (typically 3 – 8 g per portion).

IIDEA has adopted the same food categories for Inufib™ as those that were used by Imperial Sensus for Fruitafit®, with the exceptions of baby foods and meats, thus, the same methodology as that used by Environ (FDA, 2002) was used to determine the estimated daily intake of Inufib™ that would result from the proposed uses in food and beverages. Accordingly, food consumption data were based on that reported in the USDA's 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII) and its 1998 Supplemental Children's Survey (USDA, 2000).

To calculate dietary exposure to inulin, Environ combined the Dietary Risk Evaluation System (DRES) consumption estimates with food-specific inulin concentrations found in the scientific literature. Because inulin concentrations are commonly stated as ranges, calculations of both the lower and upper bound concentrations were performed. The resulting values represent lower and upper estimates of total inulin exposure from the average U.S. diet. The daily consumption estimates of foods containing inulin, the lower and upper estimates of inulin in these foods, and

[6] See "Typical Use Levels of Fructooligosaccharide, at:
http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm154400.htm

the resulting inulin intake for the average U.S. diet for various population groups are listed in Attachment 17.

### 4.3 Other Information on Human Exposure to Inulin

Human remains dating back at least 10,000 years show early uses of agave for food and fiber. The use of the agave plant has long been part of human culture dating back to the pre-colonial era. It was exported to Europe by 1520, and was mentioned as a food of Aztecs and natives in the Florentine Codex of 1580 (IOAA, 2009).

A number of fructan-rich plants have been food sources for indigenous peoples, including Dacopa, a beverage from roasted Dahlia tubers, Yacon tuber (also called Peruvian ground apple); Jerusalem artichoke tuber; Chicory root (*Cichorium intybus*); Murnong, (*Microseris scapigera*, also called the yam daisy) and Camas root. Detailed paleodietary studies demonstrate that prehistoric populations of the semi-arid northern Chihuahuan Desert consumed a wide variety of plants including *Agave lechuguilla* (agave), *Dasylirion sp.* (sotol) and *Allium drummondii* (onion). Conservative estimates of the contribution of inulin-bearing plants in the diet suggest that the average male hunter–forager from this population would have consumed about 135 g per day, and adult females about 108 g/day (based on about 20% less energy) (Leach and Solbok, 2010). Jerusalem artichokes were consumed by some populations as a substitute for white potatoes and the consumption of inulin by these populations was estimated to have reached 25 to 32 grams per day (FDA, 2002, GRN 118). Fructan-containing products derived from many of these plants are commercially available and sold online and in health food stores in the United States.

Agave plants serve as a food source in some states of Mexico, and their use predates the arrival of the Spaniards. Certain tribes learned to cook agave plants and use them as food to compensate for the lack of water in the desert. These tribes discovered that cooked agave soaked in water could ferment, producing a desirable beverage. This method was used for centuries to produce a variety of beverages from agave (Cedena, 1995). In the modern era, Kolbye et al. (1992) states that inulin and oligofructose have a "history of long-term use before 1958."

As reviewed by Roberfroid and Delzenne (1998), inulin-type fructans are present in a variety of edible fruits and vegetables in appreciable quantities. The most common sources are wheat, onions, bananas, garlic, and leek. The inulin-type fructan content of edible plants ranges from <1% to >20% of the wet weight. In populations consuming a Western-style diet, the intake of inulin-type fructans has been estimated at up to 10 grams per day (Coussement, 1999) and to range between 1and 4 g/d for the 97$^{th}$ percentile in the United States. In Europe, estimated consumption is from 3-11 grams per day (Van Loo et al. 1999; Coussement 1999). Moshfegh et al. (1999) estimated the average inulin and oligofructose ingestion in the American diet was 2.6 g, and approximately 95% of that amount was attributable to wheat and onions.

In 2011, customers of IIDEA are consuming inulin from premium agave at an average rate of 25,000 kg of per month.[7]

[7] Email correspondence from Martin A. Sanchez, MASO Consulting LLC on Sept 6, 2011

## 5.0 REVIEW OF SAFETY DATA

Human tolerance to inulin-type fructans has been thoroughly evaluated in historical and contemporary diets and in clinical studies employing bolus, short-term, and long-term exposures.

Roberfroid and Delzenne (1998) describe *Agave azul tequilana* as one of the three inulin-containing plant species that are used in the food industry; the other two being Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Cichorium intybus*). By consensus, inulin-type fructans have been classified as "nondigestible" oligosaccharides, which positively affect the composition and metabolic activity of the intestinal microflora of humans. The lactic acid producing bacteria are of particular benefit because of their ability to inhibit the growth of pathogenic bacteria and to stimulate innate and acquired immune functions. The bifidogenic effect of inulin and oligofructose is independent of chain length of and well established in different age groups (Meyer and Stasse-Wolthuis, 2009). Consensus was also reached on the human evidence for the stool regulating effect of inulin-type fructans (strong evidence) and the increase in calcium and magnesium absorption (promising evidence) with moderate supplement intake (Van Loo et al., 1999).

Human and animal studies have shown that fructans cause significant decreases in total cholesterol, triglycerides and low density lipoproteins, as previously reviewed (Roberfroid and Delzenne, 1998; Delzenne and Kok, 2001). In a review of the applications of inulin and oligofructose in health and nutrition, Kauer and Gupta (2002) summarized the effect on various human health parameters of inulin/oligofructose in the diet. In addition to the bifidogenic effect of fructans and effects on serum lipids, oligofructose and inulin relieved constipation, lowered blood glucose levels, and improved the absorption of calcium.

In experimental animals fed inulin/oligofructose, the aforementioned effects described for humans were observed, and significant inhibition of the growth of various kinds of cancerous tumors was additionally found in rats (Kauer and Gupta, 2002). Fructans in the diet have been shown to improve gut microbial ecology and enhance stool quality in companion animals, and in production livestock and poultry, they are employed to control pathogenic bacteria, reduce fecal odor, and enhance growth performance (Flickinger and Fayhey, 2002).

Gibson and Shephard (2010) evaluated the efficacy of a diet low in rapidly fermentable, short-chain carbohydrates in managing functional gastrointestinal symptoms. The diet restricted foods containing fermentable oligo-, di- and monosaccharides and polyols (FODMAP) which included foods rich in fructose, lactose, fructooligosaccharides (i.e., fructans) and galactooligosaccharides (i.e. galactans), and polyols, such as sorbitol, mannitol, xylitol and maltitol. The low FODMAP diet provided relief of global symptoms in 75% of patients with irritable bowel syndrome, and improved functional gut symptoms in patients with inflammatory bowel disease. The authors also observed that restriction of FODMAP intake potentially is detrimental to large bowel heath and might promote colorectal cancer, based on the restriction of dietary components with prebiotic effects. The low FODMAP (i.e., low fructan) diet was considered by the authors to be unsuitable for healthy individuals (Gibson and Shephard, 2010).

All nondigestible carbohydrates including inulin-type fructans may cause intestinal discomfort and possible laxative action that is dose-related as a result of fermentation in the large bowel. Roberfroid and Delzenne (1998) concluded from their review of the published data that:

> In a liquid food product, a single daily dose of 10 g will not cause a transient appearance of mild symptoms of intestinal discomfort, whereas a single daily dose of 20 g may, and the single daily dose likely to cause major discomfort in most individuals (except very resistant high-fiber consumers) is 30 g. However, if the dose is split through the day into several individual servings, symptoms of discomfort will be reduced and, in most cases, will disappear, even for total daily doses as high as 20-30 g. Liquid food products containing inulin-type fructans are always more likely to induce intestinal discomfort than solid formulations are, and the risk of an effect is reduced if the food product is consumed as part of a complete meal. Finally, it must be underscored that a small percentage (1-4%) of the population might have a higher-than-average sensitivity to these intestinal discomforts. But these highly sensitive individuals are also likely to be very sensitive to the intestinal discomfort caused by sugar alcohols, any nondigestible carbohydrates, or even fermented dairy products.

A committee of experts concluded that increased exposure to inulin and oligofructose is likely to be of negligible biological significance even for a consumer at the 90th percentile (Kolbye et al, 1992), and this conclusion has stood for almost two decades.

## 5.1 Metabolism and Gastrointestinal Tract Effects of Agave Inulin

The β(2→1) and β(2→6) linkages present in agave inulin are resistant to hydrolysis by human digestive enzymes and will pass largely intact to the colon where it is subject to fermentation by colonic microflora (Lopez et al. 2003). This fermentation results in the production of gases such as hydrogen, carbon dioxide, and methane, as well as short-chain fatty acids. The short-chain fatty acids are utilized locally as an energy source by the resident flora, taken up systemically via the colonocytes and transported to the liver for caloric utilization by the host, or excreted in the feces. Fermentation products of inulin (based on studies with chicory inulin) have been shown to be protective in different stages of cancer onset since they regulate colonic epithelial turnover and induce apoptosis in colon adenoma and cancer cell lines (Munjal et al. 2009).

### **5.1.1** Human studies

López-Velázquez et al. (2013) studied the effect of fructans obtained from *Agave tequilana* var Weber on the frequency of gastrointestinally adverse events (including changes in stool consistency and incidence of colic, abdominal distention, flatulence, and regurgitations) in infants. Six groups of approximately 100 healthy infants per group were included in the study. Three groups were fed formula containing a probiotic (0.3 g / 100 mL Lactobacillus, CUF = 107) and fructans (0.5 g / 100 mL), while one group each was fed formula containing probiotic only, formula containing no probiotic or fructans, or human breast milk. Among the three groups fed formula containing both probiotic and fructans, two groups were fed formula containing fructans with an average DP of 15 (trade name Metlos®) or 27 (trade name Metlin®) respectively, and another was fed with a fructan mixture containing both Metlos® and Metlan®. For all three groups, the mean

daily formula intake over the last two months of the six month study duration ranged from 1423 - 1510 mL/day, for an average daily fructans dose of 7.1 – 7.5 g/day during the period of maximum formula consumption. Gastrointestinal effects were evaluated via Case Report Forms once per month from 20 days to six months of age. Among infants fed formula containing the probiotic and the fructan mixture, as well as infants fed formula containing the probiotic and Metlin® only, there were no significant changes in stool consistency or increases in the incidence of colic, abdominal distention, number of daily flatulence episodes, and number of daily regurgitation episodes compared with infants that were fed only breast milk. Among infants fed formula containing the probiotic and Metlos® only, there was a significant increase in the percentage of infants with > 10 flatulence episodes per day, but there were no significant changes in stool consistency, nor were there any signficant increases in the incidence of colic, abdominal distention or number of daily regurgitations. The authors concluded agave fructans, when given under the conditions of this study, is safe for use as a nutritional supplement in infants.

The gastrointestinal tolerance of agave inulin consumption in a cohort of 29 healthy men and women aged 20-36 was studied by Holscher et al. (2014). Study participants consumed daily doses of 0, 5.0, or 7.5 g agave inulin in a single serving (administered via one chocolate chew per day) for three 21-day treatment periods separated by 7-day "washout" periods, and the severity of gastrointestinal symptoms (abdominal pain, bloating, burping, flatulence, nausea, reflux, and rumblings) as well as the consistency and ease of bowel movements was recorded daily. In addition, weekly assessments of the frequency of abdominal pain, bloading, flatulence, nausea, rumblings, and diarrhea were completed by questionnaire. In the daily assessments, the severity of symptoms was reported on a scale of 0 (absent) to 3 (severe), and in the weekly assessments, the frequency of symptoms was reported on a scale of 0 (occurs no more than usual) to 2 (occurs much more than usual). The daily assessments revealed statistically significant increases in the mean scores measuring the severity of abdominal pain, bloating, flatulence, and rumblings among the treated groups compared with the placebo control group, but the reported scores indicated mild severity, ranging from a mean score of 0.2 for abdominal pain to a mean score of 1.2 for flatulence in the high dose group. Similarly, in the weekly assessments there were statistically significant increases in the mean scores among treated groups measuring the frequency of bloating, flatulence and rumblings compared with the placebo group, but the reported scores indicated only slight increases in frequency, ranging from 0.4 for rumblings to 1.0 for flatulence in the high dose group. Stool characteristics, including number of bowel movements, ease of stool passage, stool consistency, and percent dry matter were affected by agave inulin consumption. However, the magnitudes of these effects were very small; for example, the mean number of daily bowel movements increased from 1.2 in the placebo group to 1.4 in the high dose group, and the mean stool consistency score increased from 3.4 to 3.6 in the placebo and high dose groups (respectively), with higher scores reflecting softer stool consistency. The authors concluded that a daily consumption of 5 – 7.5 g agave inulin in a single serving is generally well tolerated in adults with mild flatulence reported as the most common side effect.

### **5.1.2** *In vivo* animal studies

The physiological effects of *A. tequilana*-derived fructans in the diet of mice over 5 weeks was studied to compare them with other prebiotic fructans, including commercially available chicory-derived inulin (i.e. Raftilose®Synergyl) and fructans from the *Dasylirion* spp which is similar to

agave with respect to plant morphology, geographical distribution and pollen characteristics. Groups of eight mice were given fructan supplemented diets (10%) or standard diet (controls) for 5 weeks. Body weights and food intake were measured two times per week and 24-hour feces collections were performed three times during the course of the experiment. Blood samples were taken once per week for measurement of serum glucose, triacylglycerol cholesterol and nonesterified fatty acids. Glucagon-like peptide-1 (GLS-1) was measured in terminal portal vein blood samples. Segments of the cecum and proximal, medial and distal colon collected for mRNA and GLS-1 analysis. Full and empty cecum, liver and epididymal fat tissue were weighed, and livers were kept for histological analysis. Hepatic triacylglycerol cholesterol and nonesterified fatty acids were determined.

Total cecum weight and cecum wall weight were increased by agave fructans by 100% and 77%, respectively, suggesting increased bacterial activity and an increase in short-chain fatty acid production through fermentation by colonic bacteria. Intestinal proglucagon mRNA concentrations were increased 32% in the cecum and 20% in the medial colon and GLP-l concentration were increased 90% in the cecum and increased 2.8-fold in the proximal colon. Mouse diets supplemented with the Raftilose®Synergyl or fructans from the *Dasylirion* spp for 5-weeks also induced a higher concentration of glucagon-like peptide-1 (GLP-1) and its precursor, proglucagon mRNA, in the different colonic segments. On the basis of these findings, the authors suggested that fermentable fructans are able to promote the production of satietogenic/incretin peptides in the lower part of the gut (Urias-Silvas et al. 2008).

### **5.1.3** *In vitro* studies

An *in vitro* assessment of the prebiotic effect of fructans showed an efficient stimulation of growth of Bifidobacteria and Lactobacilli by several agave fructans including *A. tequilana* Gto (Lopez and Urias-Silvas, 2007). Fructans from *A. tequilana* exhibit a similar bifidogenic potential *in vitro* as compared with a short-chain fructan derived from chicory roots inulin (Raftilose®Synergyl).

Prebiotic properties, of fructans from *Agave tequilana* Weber var. *azul* (Predilife) in batch culture fermentation systems were compared with four commercial prebiotic brands:

> Inulin (Orafti®HP, 100% inulin/oligofructose content and 0% glucose/fructose/sucrose, Orafti);
> Synergy1 (Orafti®Synergy1, 92% inulin/oligofructose content and 8% glucose/fructose/sucrose, Orafti);
> Oligofructose (Orafti®Raftilose95, 95% inulin/oligofructose content and 5% glucose/fructose/sucrose, Orafti); and
> scFOS (Actilight 950P; Beghin Meiji, Neuilly, France).

Cellulose served as a control. Specifically, the ability to selectively increase the number of bifidobacteria and alter colonic short-chain fatty acid profiles was determined (Gomez et al., 2010). Agave inulin produced significantly increased growth of bifidobacteria and lactobacilli, similar to the effect observed for established inulin-type prebiotics derived from chicory root. Total short chain fatty acid production was also increased by agave inulin with significant increases in acetate and propionate. Among the fructan substrates evaluated, the degree of increase was similar (Gomez et al. 2010).

An *in vitro* study on the effect of *Agave angustifolia* derived fructans on the bacterial composition of homogenized human fecal samples was performed using the Simulated Human Intestinal Microbial Ecosystem (SHIME) model (Allsopp et al. 2013). Fecal microbial cultures were grown in vessels simulating the environments of the proximal, transverse, and distal colon and were supplemented with standard media for two weeks, followed by a three-week treatment period with 2 g/day *Agave* fructan. Mean counts of bifidobacteria were significantly increased ($p<0.05$) in all three vessels following the three week treatment period, relative to bacterial counts in culture samples taken following the two-week pretreatment period. Mean counts of lactobacilli were similarly increased in all three vessels following the treatment period, although the increases were not statistically significant. Mean concentrations of the short chain fatty acids propionic acid and butyric acid following the fructan treatment period also showed statistically significant increases ($p<0.05$) relative to culture samples obtained after the pretreatment period. The authors concluded that *Agave* fructans have prebiotic potential, and further commented that an increase in colonic butyrate is a desirable health effect due to its beneficial effect on colonic mucosal homeostasis and its immunomodulatory activity.

In summary, healthy adult men and women, and healthy infants showed minimal to no gastrointestinal symptoms associated with the daily ingestion of up to 7.5 g agave inulin over periods of three or six months. *In vitro* studies show that agave inulin promotes the growth of colonic microflora, which in turn produce short chain fatty acids. These results are corroborated by the *in vivo* study in mice showing increased cecum and cecum wall weights.

## 5.2 Toxicology Studies with Agave Inulin

### 5.2.1 Acute Toxicity Studies

In an acute toxicity test stated as compliant to OECD Guideline 425, 25 male Balb/c mice were exposed to single gavage doses of agave fructans derived from *Agave tequilana* Weber var. azul at concentrations of 175, 550, 1750, and 5000 mg/kg (Marquez-Aguirre et al., 2013). Following a 14-day observation period, the following parameters were assessed:

Red blood cells, hemoglobin, hematocrit, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, aspartate aminotransferase, alanine aminotransferase, glucose, creatinine, and body weight gain.

Among mice treated with a single gavage dose of 5 g/kg fructans (regardless of average DP), there was no mortality and no statistically significant changes in any of the measured hematological or blood chemistry parameters compared to untreated controls. Body weight gain was similarly unaffected. The authors further stated that treatment did not affect “general state of health,” although no further details regarding which health effects were assessed in the single-dose assay were provided.

In an acute toxicity study reported by Gracia et al. (2013), groups of 5 male and 5 female Hsd:ICR mice (4-5 weeks of age) and 5 male and 5 female Hsd:WI rats (8-9 weeks of age) were given single gavage doses of agave fructans derived from *Agave tequilana* Weber at concentrations of 17.5, 55, 175, 550, 1750, or 5000 mg/kg. The animals were treated with either a low DP (< 10) or a high DP (> 10) agave fructan preparation (trade names Metlos® and Metlin® respectively). During a 14-day observation period following treatment, body weights, mortality, clinical signs including

incidences of diarrhea were recorded; and animals were monitored for condition of the fur, eyes, mucosal membranes, and respiratory system. Fourteen days post-treatment, all animals were euthanized and the stomach, small intestine (duodenum, jejunum, and ileum), large intestine (cecum, colon, and rectum) and liver were removed, fixed, and examined. No mortality, adverse clinical observations, changes in body weight, or histopathological findings in the stomach, large intestine, small intestine, or liver were reported at any dose level. The authors concluded that ingestion of agave fructans derived from *Agave tequilana* Weber at the tested dose levels is non-toxic in rodents.

### 5.2.2 Repeated Oral Dose Studies

In the 5 week mouse study described in section 5.1.2, in which a diet containing 10% fructans from *A. tequilana* was fed for 5 weeks, the effects produced were similar to the ones already described for other inulin-type fructans, namely, a decrease in energy intake and body weight gain, and a decrease in glycaemia. Significant changes in mice receiving the *A. tequilana* fructan supplemented diet compared with the standard diet included an 11% decrease in energy intake; body weight gain was ~30% of the standard diet group. Feces excretion was increased 17% on a dry basis, and adipose tissue weight was decreased 27%. There were decreased liver weights (13%), decrease in hepatic cholesterol (7%) and triacylglycerol (11%), with no differences in liver histology. GLP-l concentration measured in the portal vein was increased 1.5-fold relative to controls. Serum glucose concentration was reduced 15%, and serum cholesterol ~20% (Urias-Silvas et al. 2008).

Marquez-Aguirre et al. (2013) studied the effect of the degree of polymerization (DP) and a demineralization processing of agave fructans on body weight gain and gut bacterial profiles of obese mice. Seventy male C57/BL/6 mice (9 weeks old at study onset) were fed a high-fat diet to induce obesity, and given daily gavage doses of 5 g/kg body weight agave fructans derived from *Agave tequilana* Weber var. azul for a 12-week period. Among the treated groups, one group received agave fructans with a DP < 10, and another group received agave fructans with a DP > 10. Additional groups received total agave fructans with or without demineralization by ion exchange chromatography. At the end of the treatment period all animals were sacrificed and gross pathological examination was performed on all organs. Blood serum was analyzed for total cholesterol and triglycerides and fat tissue was measured by the excision and weighing of white adipose tissue. In addition, all mice were observed throughout the duration of the treatment period for mortality, body weight effects, and clinical signs, although further details were not provided. Quantification of *Lactobacilli* and *Bifidobacteria* in mice fecal samples recovered from the colon immediately following sacrifice was performed by real-time PCR.

Animals given the high fat diet and treated with low-DP agave fructans had significantly reduced body weight gain, fat tissue, and total serum cholesterol compared with control animals given the high fat diet with no agave fructans. Conversely, animals given the high fat diet and treated with high-DP agave fructans or total agave fructans did not show statistically significant changes in body weight gain, fat tissue, or total cholesterol, but did have significantly reduced triglycerides compared with control animals given the high fat diet with no agave fructans. Evaluation of the intestinal content of obese mice treated with the demineralized total agave preparation revealed a bifidogenic effect, defined as an increased relative abundance of *bifidobacterium* to *lactobacillus*

when compared with control animals given either the high fat diet or standard diet with no agave fructans. The bifidogenic effect was not observed in the groups given the low-DP, high-DP or total agave fructan preparations. The authors concluded that agave fructans with low DP can prevent body weight gain and fat tissue accumulation associated with a high fat diet without bifidogenic activity. Although the results of the pathological examinations and clinical observations were not specifically described, it can be inferred that consumption of 5 g/kg fructans derived from *Agave tequilana* Weber var. azul was not associated with any overtly adverse health effects in mice since none were reported.

In summary, the effects of agave inulin in rats and mice on body weight gain, fecal bulk, and glucose and/or lipid metabolism provide evidence that agave inulin acts in a manner similar to other non-digestible polysaccharides. No adverse effects were identified following acute or repeated oral dosing.

### 5.3 Genotoxicity of Agave Inulin

Agave inulin has been shown to be non-mutagenic *in vitro*. In a bacterial reverse mutation assay conducted on *S. typhimurium* strains TA98, TA100, and TA102, agave fructans derived from *Agave tequilana* Weber var. azul at a concentration of 800 µg/plate did not significantly increase the frequency of mutations relative to negative controls, both with and without metabolic activation with Arochlor-1254 induced S9 mixture (Marquez-Aguirre et al., 2013). The study protocol was stated as compliant to methods described in Maron and Ames (1983) but deviated from current standardized guidelines (including OECD Guideline 471 and FDA Redbook) in the following ways: (1) testing did not include at least five strains of bacteria, including *S. typhi* TA1535 and TA1537 or TA97a or TA97 in addition to *S. typhi* strains TA98, TA100, and TA102 and (2) for noncytotoxic substances a maximum concentration of 5 mg/plate is recommended, which is below the 800 µg/plate concentration used in the study. Although this study does not conform to standardized guidelines and no rationale was provided for the noted deviations, based on structure-activity considerations, agave inulin is not expected to interact with DNA, and the mutagenic potential is expected to be negligible.

*In vivo* chromosomal aberration and micronucleus assays were conducted with Hsd:ICR mice by Gracia et al. (2013) to evaluate the genotoxicity of agave fructans derived from *Agave tequilana* Weber. A low DP (< 10) and a high DP (> 10) agave fructan preparation (trade names Metlos® and Metlin® respectively) were used in the study. Groups of male mice 4-5 weeks of age (5 per treatment group) were given intraperitoneal injections of 143, 357.5, or 715 mg/kg of Metlin® or Metlos®, while two additional groups were given Mitomycine-C or phosphate buffer solution (PBS) as a positive and negative control, respectively. Twenty-four hours after treatment, 5 µl of peripheral blood from the tail vein was collected from each animal. Subsequently, the animals were euthanized and bone marrow was extracted from the femur of each animal. For the chromosome aberration study, 100 bone marrow cells in metaphase from each animal were scored for alterations in the chromosomes and chromatids. For the micronucleus assay, erythrocytes from tail vein blood were stained and examined for frequencies of micronucleated polychromatic erythrocytes in a fluorescence microscope. The chromosome aberration assay was stated by the authors as compliant to OECD Guideline 475 and EPA OPPTS 870.5385. No specific guideline was cited by the authors for the micronucleus assay, although based on the methodology described

it is compliant to OECD Guideline 474, although no rationale for the selected doses was provided. In the chromosome aberration assay, the number of cells with deletions, fragments, translocations, or gaps was not significantly increased among the Metlin® and Metlos® treated groups compared to negative controls. Similarly, in the micronucleus assay, the mean frequency of micronuleated cells was not significantly increased by treatment with Metlin® and Metlos® at any dose, compared with the negative control group. The authors concluded that agave fructans derived from *Agave tequilana* Weber is non-genotoxic in mice.

### 5.4 Systemic Effects of Agave Syrup

Figlewicz et al. (2009) evaluated the effect of iso-caloric solutions of agave syrup (12.5%), fructose (12.5%), high fructose corn syrup (HFCS; 15%), as well as HFCS with the appetite suppressant Hoodia, and the noncaloric sweetener Stevia (12.5%), in young adult male Albino rats. Sweeteners were supplied in drinking water, 3 nights per week for 10 weeks to groups of 10 rats each and 10 controls were given drinking water with no added sweetener. Food intake, beverage intake and body weight were determined. At the end of the 10 weeks, rats were assessed for glucose tolerance, and the following terminal blood chemistries were measured:

> serum cholesterol, triglycerides, VLDL, HDL, and LDL, alanine-amino-transferase, serum bilirubin, and serumalbumin, serum creatinine, alkaline phosphatase, blood urea nitrogen (BUN). Neuroendocrine anorexic signaling was assessed by measurement of plasma leptin and peptide YY (PYY). Serum levels of (IL6, MCP1, TNFα, and IL-1b) as markers of inflammation were determined.

Body adiposity and liver histology were also assessed.

Chronic ingestion of sweeteners did not result in changes in final body weight, total weight gain, or retroperitoneal fat pad weight of the rats. There was no difference in PYY levels among all groups and no overall effect on plasma leptin levels indicating no effect on neuroendocrine signals of satiety or adiposity.

For all groups consuming the calorie containing sweeteners, levels of fasting lipids (triglyceride, total cholesterol, and VLDL) were elevated compared with the drinking water controls. In the agave syrup group, serum triglycerides and VLDL were increased significantly ($p<0.05$). Mean levels of the inflammatory markers, MCP1, TNFα, and IL-1b, did not differ among Stevia-, agave syrup-, fructose-, HFCS-, or water-consuming rats.

There was no hepatic steatosis in any of the animals, as assessed with Sudan black staining, with no between-group differences. Hepatic architecture was preserved in all animals with no significant inflammatory infiltrates, and no fibrosis. Serum albumin and bilirubin levels were similar between the sweetener groups and the control (water) group. ALT levels in the agave syrup consuming animals were normal, and lower than the levels in the pure fructose group. The authors suggested that beneficial antioxidant nutrients, trace elements, or phytochemicals may be present in the agave syrup to account for the difference observed in agave and fructose groups.

## 5.5 Toxicology Studies with Fructooligosaccharides

In a critical review of the animal toxicology data and clinical studies of inulin-type fructans Carabin and Flamm (1999) concluded that these fructans have not shown evidence of mortality, morbidity, target organ toxicity, reproductive or developmental toxicity, mutagenicity or carcinogenicity. The authors considered toxicological studies performed with synthetic fructooligosaccharides (average DP = 4), to be predictive of the effects of naturally occurring inulin and oligofructose since the substances are chemically similar entities with like nutritional properties.

Briefly, the rat oral $LD_{50}$ for fructooligosaccharides was determined to be greater than 9 g/kg. In a rat 6-week toxicity study, there was no treatment-related toxicity in any of the fructooligosaccharides-treated groups up to a dose of 4.5 g/kg administered by gavage, as assessed by blood parameters, and histopathology of the liver, pancreas, adrenal glands, kidneys, brain, cerebellum, heart, lungs, spleen, pituitary gland, and testes. A slight increase in body weight was observed in the two highest dose groups compared to controls. There were no deaths during the study, and the only treatment-related change observed at necropsy was swelling of the appendix in fructooligosaccharides treated rats (Takeda and Niizato, 1982).

In a rat 6-week feeding study, test diets enriched with 5 or 10% fructooligosaccharides, or control diets containing sucrose, glucose, or sorbitol were evaluated. In the fructooligosaccharide groups there were decreased body weights, a reduction in cholesterol, and swelling of the appendix, while incidences of kidney and hepatic pathology were similar across control and test groups. It was concluded that fructooligosaccharides showed no toxicity compared with existing sugars commonly used in the food supply. The study also demonstrated that blood glucose levels were not raised significantly by a single oral dose administration of fructooligosaccharides, and it was therefore concluded that the reduction in body weight was due to the low caloric content of fructooligosaccharides (Takeda and Niizato, 1982).

In a 2-year carcinogenicity study with male and female Fischer 344 rats (Clevenger *et al.*, 1988), test animals were given fructooligosaccharides with their diet at concentrations of 0, 8000, 20,000, and 50,000 ppm (equivalent to 0, 341, 854, and 2170 mg/kg-day, respectively, for male rats and 0, 419, 1045, and 2664 mg/kg-day, respectively, for female rats). The study revealed that the long term ingestion of fructooligosaccharides produced no significant dose-related effects on body weight, food consumption, survival, growth, hematology, blood chemistry, or organ weights, nor did the treatment affect the incidence of neoplasms.

Henquin (1988) reported on the lack of developmental toxicity of fructooligosaccharides. Twelve female Wistar rats with a copulation plug were fed a diet containing 20% FOS from day 1 to 21 of gestation. A separate group of 17 female Wistar rats with a copulation plug were fed a control diet for the same period of time. A reduction in body weight gain of the pregnant rats was attributed to a lower caloric value for fructooligosaccharides, decreased intake of food, and/or diarrhea observed in the first week and softer stools in the second and third weeks. There were no significant effects on the course of pregnancy or on the development of fetuses and newborns.

Maternal and developmental toxicity was also evaluated by Sleet and Brightwell (1990) in the rat (strain Crl CD (SD) BR) following administration of fructooligosaccharides in the diet during gestation. Four groups of 24 to 27 pregnant females were pretreated with fructooligosaccharides at a dietary level of 4.75%, from day 0 to 6 postcoitum, in an attempt to avoid diarrhea observed with earlier studies. A fifth group received a fructooligosaccharides-free diet throughout the entire study. On postcoital day 6, the fructooligosaccharides pretreatment diet was replaced, with each group receiving the following diets until Day 15: fructooligosaccharides-free diet, 5, 10, and 20% fructooligosaccharides. Pregnant females were placed on a fructooligosaccharides-free diet from Day 15 until sacrifice on Day 20. Dietary supplementation with fructooligosaccharides at concentrations up to 20% did not cause adverse effects (e.g., diarrhea) or negatively affect the pregnancy outcome or *in utero* development of the rat. The only treatment-related effect was the alteration in the body weight of the dams, with a moderate reduction seen in the 20% fructooligosaccharides group.

Fructooligosaccharides exhibited no genotoxic activity in three assays conducted with and without metabolic activation, which included the bacterial reverse mutation assay with *Salmonella typhimurium* (strains TA1535, TA1537, TA1538, TA98, and TA100) and *Escherichia coli* WP2 *uvr* A, the L5178Y mouse lymphoma TK6 mammalian cell mutation assay, and an unscheduled DNA synthesis assay in human epithelioid cells (Clevenger *et al.*, 1988).

### 5.6 Toxicological Studies with Carboxymethyl Inulin

Johannsen (2003) reviewed the toxicological properties of carboxymethyl inulin, a material used as an anti-scalant in food processing applications that is synthesized by carboxylation of a chicory-derived inulin. Several studies conforming to international test guidelines were reviewed. In brief, a rat 4-week toxicity study by the gavage route showed no treatment-related effects in body weight, food consumption, mortality, hematology, clinical blood chemistry, organ weights or gross or microscopic pathology up to the highest dose of 1000 mg/kg-day. Females in the 1000 mg/kg-day group showed a modest increase in motor activity, however, this finding was not considered toxicologically significant. A guinea pig Magnusson–Kligman maximization test showed no evidence of dermal sensitization with carboxymethyl inulin. It was also not genotoxic in either bacterial reverse mutation assays conducted with Salmonella strains TA1535, TA1537, TA98 and TA100 or in Escherichia coli WP2uvrA, and did not induce chromosomal aberrations Chinese hamster ovary cells *in vitro.*

### 5.7 Dietary Studies with Fructans (from sources other than *Agave tequilana* Weber var. *azul*)

#### **5.7.1** Animal studies

Rao et al. (1965) conducted 6-week feeding studies in albino rats to evaluate the effects of polyfructosans from the stems of the *Agave vera-cruz* plant as compared with inulin (Merck & Company). Test diets were supplemented with 5% agave fructosans or 5% inulin at the expense of starch and controls were given basal diets (n=8). Food intake, body weights, total cholesterol in liver and plasma, fecal steroids, and excreted bile acids were determined in rats. Body weight gain was lower in the groups given fructosan (10%) and inulin (13%) diets compared to controls.

Fructosan and inulin were largely unutilized based on an increase in fecal bulk in rats given the test diets relative to controls. Mild diarrhea was observed in the rats fed agave fructosan. Highly significant reductions in plasma cholesterol levels were observed in fructosan (35%) and inulin (22%) treated groups, and hepatic cholesterol was reduced ~10% in both groups. The average fecal sterol excretion was 16.3 mg/day in the fructosan diet group compared with 10.4 and 11.2 mg/day in the basal diet and inulin diet, respectively. Mean fecal excretion of bile acids was unchanged in the fructosan group (16.8 mg/day) compared with controls (17.0 mg/day) and reduced to 11.9 mg/day in the inulin diet group, however, the difference was not significant.

Buddington et al. (2002) reported studies with B6C3F1 mice in which diets containing nondigestible β-fructans provided protection against various health challenges. In the control diet, the sole source of fiber was 100 g cellulose/kg; in the test diets, the cellulose was replaced with oligofructose (Raftilose P95; Orafti, Tienen, Belgium) or inulin (Raftiline HP; Orafti, Tienen, Belgium). These levels of dietary fiber (10%) are comparable to levels of fiber commonly recommended for human intake. Test and control diets were fed for a 6-week period before the challenges to allow for full adaptation of the gastrointestinal tract ecosystem, and the diets continued to be fed throughout the challenge period. Concurrent studies with B6C3F1 mice verified that diets containing 100 g/kg inulin or oligofructose increased the densities of lactic acid-producing bacteria.

To assess enteric defense functions, mice that were fed the control and the two experimental diets for 6 weeks (25 per diet) were inoculated orally with the enteric pathogen *Candida albicans* and examined 7 days later; or mice were injected subcutaneously with 1,2-dimethylhydrazine (20 mg/kg) once per week for 6 weeks, sacrificed 4 weeks after the last injection and evaluated for aberrant crypt foci in the colon as in early indicator of preneoplastic lesions.

To assess systemic defense functions, mice were given an intraperitoneal injection of virulent strains of the pathogens *Listeria monocytogenes* or *Salmonella typhimurium,* and survival was determined over a two week period. Alternatively, 25 mice from each diet group were injected subcutaneously with $5 \times 10^4$ B16F10 tumor cells and the number of nodules on the surface of the lungs was recorded 28 days later.

Yeast densities in the small intestine of mice given *Candida albicans* were reduced ~ 50% ($P < 0.05$%) in the test diets containing fructans compared to the controls. The incidences of aberrant crypt foci in the distal colon after exposure to dimethylhdrazine for mice fed inulin (53%) and oligofructose (54%) were lower than in control mice (76%; $P < 0.05$). Mice systemically infected with either *L. monocytogenes* or *S. typhimurium* and fed the diets containing fructans had lower mortalities compared with mice given the control diet. The survival rate of mice infected with *L. monocytogenes* was 70% among the controls and was significantly greater ($P < 0.05$) in both the inulin group (100%), and the oligofructose group (88%). Survival was also improved ($P < 0.05$) in the mice infected with *S. typhimurium* by the inulin diet (40% survival) versus controls (20% survival), with an intermediate survival of ~25% in the oligofructose group. There was no effect of the fructan diets on the incidence of lung tumors after injection of the B16F10 tumor cells.

Under the conditions of this study, feeding inulin or oligofructose to mice prior to enteric challenges and systemic bacterial infections resulted in an increased host resistance to the

challenges. The authors concluded that gastrointestinal tract bacteria remain responsive to long-term feeding of fructan prebiotics.

Femia et al. (2002) studied the effect of chicory-derived fructans on azoxymethane-induced colon cancer in male F344 rats. Male rats were given food with 10% Raftilose-Synergy1® (inulin enriched with oligofructose) with or without probiotic treatment. Controls were feed a standard diet. Ten days after beginning the diets, rats were treated with azoxymethane (15 mg/kg s.c. two times); dietary treatments were continued for the entire experiment. Thirty-one weeks after azoxymethane injections, rats in the fructan diet groups had a significantly lower ($P < 0.001$) number of tumors (adenomas and cancers) than controls. Colorectal tumors/rat were as follows: controls, 1.9 ± 1.7; fructan diet, 1.1 ± 1.1; probiotic treatment, 2.2± 1.4; and combined fructan and probiotic treatment, 0.9 ± 1.2. Short-chain fatty acids in the cecum were higher ($P < 0.001$) in the groups given fructans. Colonic proliferation was lower in the fructan diet group but there was no change in the rates of apoptosis as compared with controls. Expression of glutathione *S*-transferase placental enzyme pi type and inducible nitric oxide synthase were depressed in the tumors from rats receiving fructan-containing diets relative to controls. Under the conditions of this study, the administration of prebiotic fructans in the diet decreased azoxymethane-induced carcinogenesis. A combination of mechanisms, involving an increase in short-chain fatty acid production, lower proliferative activity and expression of enzymes involved in the pathogenesis of colon cancer were proposed by the authors as contributing factors.

Dávila-Céspedes et al. (2014) studied the potential protective effect dietary consumption of fructans derived from *Agave salmiana* in Wistar rats (n=36) against azoxymethane-induced carcinogenesis. Two intraperitoneal injections of 15 mg/kg azoxymethane were administered with a one week interval between injections, and administration of either standard diet or diet containing 10% fructans derived from either *A. salmiana* or *C. intybus* continued for a 13-week period following the second injection. In an effort to acclimate the study animals to experimental conditions, administration of the 10% fructan diet began prior to the azoxymethane injections, but the duration of this acclimation period was not reported. The authors reported that the number of aberrant crypt foci found in the colon of azoxymethane treated rats fed *A. salmiana* fructans was significantly lower than the control group fed a standard diet with administration of azoxymethane ($p<0.05$); however, this number was three times larger than the number of aberrant crypt foci observed in the azoxymethane treated rats with fed *C. intybus* fructans. The authors speculate that this difference may be due to differences in the chemical composition of fructans derived from these two sources, although the authors also acknowledged that the purity of the *A. salmiana* fructans used in the study was not determined and did not undergo the same purification process as the *C. intybus* fructans. Thus, this study suggests that administration of unpurified *A. salmiana* fructans or C. intybus fructans in the diet have anticarcinogenic potential in the rat.

In a 28-week dietary study, Hijová et al. (2013) evaluated the effect of oligofructose-enriched inulin derived from chicory root (Orafti Synergy1) on bacterial activity, cytokine levels, and the expression of chemopreventive markers cyclooxygenase-2 (COX-2) and nuclear transcription factor kappa beta (NFkB) in Sprague-Dawley rats exposed to a cancer-causing agent. To induce colon cancer development, two groups of ten rats were injected subcutaneously with once weekly doses of 21 mg/kg dimethylhydrazine (DMH) for the first five weeks of the study, while being fed either standard diet (DMH-only) or diet composed of 8% inulin (DMH+inulin). After the 28-week

treatment period animals were sacrificed, body weights were measured, blood samples were analyzed for interleukin concentrations, feces were recovered for microbial analysis, and tissue sections from the jejunum and colon were taken for assessment of cytokine levels, COX-2, and NFkB. Mean body weight gain increased by 20% and 28% in the DMH-only and DMH+inulin groups, respectively. DMH+inulin rats had significantly increased fecal levels of alpha galactosidase ($p<0.01$) and significantly decreased fecal levels of beta-glucuronidase ($p<0.01$) relative to DMH-only rats. DMH+inulin rats also had significantly reduced serum levels of the cytokines IL-2 ($p<0.001$) and TNF alpha ($p<0.05$); and had a significantly increased level of serum IL-10 ($p<0.001$) relative to the DMH-only group. Similarly, DMH+inulin rats had significantly decreased levels of jejuna IL-2 and TNF alpha ($p<0.001$), and increased IL-10 ($p<0.001$) relative to DMH-only rats. DMH+inulin rats had significantly reduced numbers of COX-2 and NFkB positive cells in the colon ($p<0.001$ and $p<0.05$, respectively). The authors attributed the increased alpha galactosidase in the DMH+inulin group to an increased amount of lactobacilli in the gut, and further attributed the decreased levels of COX-2, IL-2, and TNF-alpha and increased IL-10 in the DMH+inulin rats compared to the DMH-only rats to an anti-inflammatory and immune enhancing effect.

The effect of fructans extracted from onion (*Allium cepa* L) was studied in male F344 rats in a 4-week feeding study (Roldan-Marin et al. 2009). Groups of 8 rats were given diets containing 7% of the fructan extract or control diets. A semiquantitative size distribution analysis of the fructans in the extract indicated that > 90% had ten fructose residues or less, and > 60% had five residues or less, with very small amounts of longer chain fructans present. There was a significant decrease ($P<0.05$) in the hemoglobin concentration in treated rats compared with the rats in the control group, consistent with a previously noted anemia caused by onions fed to rodents. Antioxidant enzyme activities were measured in erythrocytes and in liver. There was a significant increase ($P<0.05$) in glutathione reductase and glutathione peroxidase activities in erythrocytes of rats fed the test diet while hepatic glutathione peroxidase activity was significantly decreased ($P<0.01$) and hepatic glutathione reductase activity was unchanged compared with controls. There was no DNA damage as measured in liver and leukocytes by the comet assay. There was no significant difference in gastrointestinal transit time in the test diet group compared to the control group. The test diet had prebiotic effects as evidenced by decreased pH, increased butyrate and propionate production and an increase in the cecal microbiota enzyme activities, β-glucosidase and β-glucuronidase. Hepatic gene expression of Gr, Gpx1, catalase, 5-aminolevulinate synthase and AD(P)H:quinone oxidoreductase were not altered in the test group.

In a 6-week feeding study, the effect of fructans from three sources (*Agave angustifolia, Helianthus tuberosus,* and *Cichorium intybus*) was studied in diabetic and obese Wistar rats (sex not specified). In two separate experiments (one consisting of non-obese rats and the other consisting of obese rats), diabetic rats and non-diabetic rats (groups of four rats each) were assigned to four treatment groups: a control group and three treatment groups in which fructans from each of the respective three sources were administered in feed at a concentration of 15%. This concentration corresponds to approximately 9 g fructans per kg-day for all non-obese diabetic and non-diabetic groups and 7 g fructans per kg-day for all obese diabetic and non-diabetic groups, accounting for the mean daily food intakes and mean body weights reported in the study (Rendón-Huerta et al. (2012). Fecal bacterial concentrations and the incidence of liver steatosis were also assessed. Among all the treated non-obese and obese groups (both diabetic and non-diabetic) mean

total body weight gain and daily feed intake was reduced over the 6-week treatment period, with the effect reported as statistically significant in the non-obese rats ($p<0.05$) but not the obese rats. Body weight reductions in agave fructan fed rats were modest (<6% relative to the corresponding controls) and are not considered adverse. Also regardless of obesity or diabetic status, fecal concentrations of *Lactobacillus* and *Bifidobacterium* were significantly increased compared with controls ($p<0.05$), while fecal concentrations of *Clostridium* were significantly reduced compared with controls ($p<0.05$) among the treated diabetic groups but not the treated non-diabetic groups. Fecal concentrations of *E. coli* were unaffected in all treatment groups regardless of obesity and diabetic status. Among the non-obese groups treated with fructans derived from *H. tuberosus* and *A. angustifolia*, blood glucose and low density lipoprotein (LDL) concentrations were significantly reduced ($p<0.05$) in diabetic rats but were unaffected in non-diabetic rats. Conversely, among obese rats mean blood glucose was significantly lowered ($p<0.05$) in non-diabetic rats but not diabetic rats. LDL, HDL and cholesterol concentrations were significantly less that controls only among obese diabetic rats treated with fructans derived from *A. angustifolia.* The incidence of liver steatosis was unaffected by fructan consumption among non-diabetic rats regardless of obesity status; however, among diabetic rats, the incidence of grade 1 liver steatosis increased ($p<0.05$) while the incidence of grades 2 and 3 liver steatosis decreased relative to controls ($p<0.05$) in all treatment groups. The authors concluded that while consumption of fructans modulated the intestinal bacterial profile in a consistent manner regardless of diabetic and obesity status, potential beneficial effects such as reductions in blood glucose and cholesterol, as well as attenuations in the incidence of liver steatosis were often dependent upon diabetic and obesity status and tended to be most pronounced among mice treated with fructans derived from *Agave angustifolia*. The authors further speculate that differences in mean fructan DP among the three sources of fructans used in the study contributed to the differences in magnitude and consistency of the effects among the sources.

### **5.7.2** Human experience with dietary fructans

Clinical information on the intake and tolerance of fructans in humans was reviewed and summarized by Carabin and Flamm (1999). Effects that potentially develop from the use of fructans in the diet, i.e., flatulence, bloating, abdominal distention, and rumbling, are the same as those symptoms associated with the intake of fruits and vegetables, and are related to the influence of fructans on osmotic colonic pressure. The effect of inulin, oligofructose and synthetic fructose oligosaccharides on the gastrointestinal tract differ as a function of their chain lengths. In this regard, smaller molecules have a higher osmotic colonic pressure, and slower fermenting compounds are more easily tolerated than faster fermenting compounds. The potential for osmotic diarrhea is greater with fructooligosaccharides having an average DP of 3 than with inulin having an average DP of 10. Likewise, fructooligosaccharides induce less diarrhea than the disaccharide maltitol and significantly less than the monosaccharide sorbitol (Takeda and Niizato, 1982). Inulin, as a slower fermenting compound, has better gastrointestinal tolerance than fructooligosaccharides or oligofructose, and similarly, agave inulin can be expected to be better tolerated than the shorter chain molecules with respect to gastrointestinal symptoms.

The available data on inulin and oligofructose have demonstrated no evidence of toxicity based on animal and clinical evidence. Signs of gastrointestinal intolerance are observed with intakes above 20–30 g; however fructans are better tolerated when given with solid food and when given in divided doses throughout the day. Carabin and Flamm (1999) concluded that inulin-type fructans

are safe for human consumption under intended conditions of use as a dietary fiber, and that up to 20 g/day of inulin and/or oligofructose is well tolerated.

The gastrointestinal tolerance of native chicory inulin and its shorter chain length oligofructose were evaluated at 5 and 10 g doses compared to a placebo control (Bonnema et al. 2010). Twenty-six healthy men and women ages 18 to 60 years participated in the study. Healthy subjects with no history of gastrointestinal conditions consumed diets with typical amounts of fiber. The two inulin fibers tended to increase gastrointenstinal symptoms mildly. Most frequently reported symptoms were flatulence followed by bloating. The 10 g dose of oligofructose substantially increased GI symptoms compared to control. Doses up to 10 g/day of native chicory inulin and up to 5 g/day of oligofructose were well-tolerated in healthy, young adults.

Tarini and Wolever (2010) studied the effects of inulin on postprandial glucose, insulin, short-chain fatty acids, free fatty acids, and gut hormone responses in healthy subjects. Overnight-fasted healthy subjects ($n$ = 12) were studied for 6 hours after consuming 400 mL drinks, containing 80 g high-fructose corn syrup (80HFCS), 56 g HFCS (56HFCS), or 56 g HFCS plus 24 g inulin (Inulin), using a randomized, single-blind, crossover design. A standard lunch was served 4 hours after the test drink. Glucose and insulin responses after Inulin did not differ significantly from those after 80HFCS or 56HFCS. Serum acetate, propionate, and butyrate were significantly higher after Inulin than after HFCS drinks from 4–6 h. Free fatty acids fell at a similar rate after all 3 test drinks, but were lower after Inulin than after 56HFCS at 4 h (0.40 ± 0.06 vs. 0.51 ± 0.06 mmol/L; $p < 0.05$). Compared with 56HFCS, Inulin significantly increased plasma glucagon-like peptide-1 concentrations at 30 min, and reduced ghrelin at 4.5 h and 6 h. The authors concluded that inulin reduces postprandial free fatty acid rebound and reduces the serum ghrelin response after a subsequent meal, events associated with increased colonic short-chain fatty acid production.

Only two cases of anaphylaxis to inulin in food have been published (Gay-Croisier, 2000 and Franck et al. 2005), indicating that allergy to inulin is extremely rare given its widespread presence and use in the food. In the case reported by Franck et al. (2005) a 50-year-old woman with a past history of allergy to artichoke presented with two episodes of immediate allergic reactions, one of which was a severe anaphylactic shock after eating two types of health foods containing inulin. Inulin (Raftiline®HP) was included in both products for its bifidogenic effect: 0.38 g in one biscuit and 2.5 g in the yoghurt. Specific IgE to an inulin-protein compound was identified using dot blot and dot blot inhibition techniques, suggesting possible inulin binding to food proteins during heating. The authors concluded that consumers of health foods containing Raftiline with any history of allergy to artichoke or endive should be warned (Franck et al. 2005).

### 5.8 Effects of Other Constituents of the Agave Plant

There is much known about the toxicology of other consistituents of the agave plant. As discussed in Sections 3.3 and 3.5, these constituents were not detected. A summary of the available toxicology studies on the non-carbohydrate constituents of the agave plant is included in Appendix 9.2

# 6.0 GRAS SAFETY EVALUATION

## 6.1 GRAS Criteria

FDA defines "safe" or "safety" as it applies to food ingredients as:

> "…reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance."[8]

Amplification is provided in that the determination of safety is to include probable consumption of the substance in question, the cumulative effect of the substance, and appropriate safety factors. It is FDA's operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that

> **"…**General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food."[9]

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following components:[10]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as the National Academy of Sciences.

The apparent imprecision of the terms "appreciable", "at the time" and "reasonable certainty" demonstrates that the FDA recognizes the impossibility of providing absolute safety, in this or any other area (Lu 1988; Renwick 1990, Rulis and Levitt, 2009).

## 6.2 Analysis of Agave Inulin

Evaluation of the safety of Inufib™, incorporated into foods as a bulking or bifidogenic agent, was accomplished through a review of the extensive database on the safety of inulin and related β(2→1)

[8] See 21 CFR 170.3(i).
[9] See 21 CFR 170.30(a).
[10] See Footnote 1.

fructans, oligofructose and fructooligosaccharides. This review included the production process, gastrointestinal fate, animal studies and human exposure.

The safety of Inufib™ is predicated on multiple factors which include:

- The similarity of the composition of agave inulin to other plant fructans,
- The expected levels in the diet of fructans and fatty acids (~5 to 30 μg/g) from agave inulin; and
- The safety and tolerability of agave inulin as demonstrated by animal studies and human experience,

In addition, foods and beverages made from the ***Agave tequilana*** **Weber var. azul** plant have a substantial history of human consumption. The most well known food industry use of this plant is for the production of tequila which is the distilled product of fermented inulin-containing agave juice.

**6.2.1** Composition of agave inulin and similarity to other plant-derived fructans

The Panel has reviewed the manufacturing procedure, food grade specifications and batch analyses for Inufib™ and agrees that IIDEA's manufacturing and analytical procedures provide ample documentation that the product is food grade.

Inulin, oligofructose, and fructooligosaccharide are chemically similar entities demonstrating like nutritional properties. Their chemical and nutritional similarities are due to the basic structure similarities: 1.) β(2 →1) linkage of fructosyl units which sometimes end with a glucosyl unit, and 2.) to their common metabolic pathway (that is fermentation by the microflora of the colon). The only difference between inulin/oligofructose and fructooligosaccharide is in the degree of polymerization (which is the number of individual monosaccharide units which make up the molecule) (Carabin and Flamm, 1999).

The Panel reviewed the composition of chicory inulin which has attained GRAS status (FDA, 2002), and notes that fructans extracted from chicory roots and agave stems contain nearly identical quantities of inulin (~90%) and combined mono and disaccharides consisting of mainly sucrose, glucose and fructose (~10%). Moreover, fructan-containing plant species are commonly eaten as vegetables (e.g., asparagus, garlic, leek, onion, artichoke, Jerusalem artichoke, scorzonera, chicory roots (Van Loo et al., 1995). The types of linkages in these fructans vary quantitatively but are qualitatively similar. The Panel considers the fructan composition of agave inulin to be sufficiently similar to other edible fructans, including chicory inulin, and agrees that it is reasonable to conclude that the same consumption limitations placed on the related fructans should apply to agave inulin.

In Inufib™ the concentrations of terpenes and saponins are below 0.1 ppm (Attachment 1 "Saponins and Terpenes"), and saponins were not detected at levels as low as 7 ppb (Attachment 2 "Letter saponins Ext Lab). For comparison with data from the literature, when three species of agave plants, including *A. tequilana* Weber var. *azul*, were characterized by Pena-Alvarez et al. (2004), the concentration of fatty acids in the stem tissue of *A. tequilana* was determined to be

985µg/g; or approximately 0.1%. Additionally, thirty-two terpenes/terpenoids types were detected in the piñas tissue but were not quantified because they were found at extremely low concentrations (Pena-Alvarez et al., 2004). The principal terpene in the piñas was linalool which is ubiquitous in edible fruits, herbs and spices and is used as a food flavoring agent, with estimated consumption from these sources of 40 to 140 µg/kg-day (OECD, 2002). The analysis of tequila by Ávila-Fernández et al. (2009), showed a combined terpene/terpenoid content of 1-3 mg/L and linalool content of 0.5 mg/L. The estimated potential concentration of linalool in dried agave inulin, based on its measurement in tequila, is ~0.4 mg/kg (see Section 9.1.4 for derivation) (~0.4 ppm). If the dried inulin was consumed at 20 g per day, the ingested amount of linalool from this source would be 7.6 µg/day or <0.13 µg/kg-day for a 60 kg individual. Linalool is a moderate skin irritant but has a low sensitizing potential. It is neither mutagenic nor carcinogenic. It is excreted relatively rapidly and there is no tendency for bioaccumulation. The overall toxicity of linalool is low with a rat oral $LD_{50}$ of 2790 mg/kg and a 4-week rat oral gavage NOAEL of 160 mg/kg/day (OECD, 2002). It is concluded that the estimated concentration of linalool in the pin a tissue is far below concentrations posing any concern.

The bioactive saponins that have been isolated from the leaves, roots, and fruit, of agave (Appendix Table A-1) have not been detected in the piñas or in agave inulin.

### **6.2.2** Safety of and tolerance to agave inulin

Human tolerance to inulin has been thoroughly evaluated in historical and contemporary diets and in clinical studies employing bolus, short-term, and long-term exposures (FDA, 2002). Data reviewed on both oligofructose and fructooligosaccharides indicate that ingestion of up to 40 grams inulin/day is safe and well tolerated (Grühn, 1994). Any adverse effects that occur are expected to be gastrointestinal in nature and are not expected to endanger the health of the individual. Repeated daily ingestion of agave inulin was well tolerated in adults over three 21-day periods when evaluated at doses of 5.0 or 7.5 g per day (Holscher et al. 2014). Other studies have suggested that up to 70 grams of inulin per day, consumed as a regular part of the diet, may be well tolerated (FDA, 2002).

The safety and tolerance of fructooligosaccharide ingestion by infants is documented in a Japanese nationwide survey of 20,742 infants ingesting formula containing 0.32 g/100 mL (Yamamoto and Yonekubo, 1993). This results in an estimated mean and 90th percentile consumption of 3.0 and 4.2 grams fructooligosaccharides/day. A higher level of agave inulin was also well tolerated in infants when administered daily via infant formula for > 5 months at a concentration of 0.5 g / 100 mL or approximately 7.5 g/day (López-Velázquez et al. 2013). The estimated daily intake (EDI) of inulin from all of the proposed uses of Inufib™ for infants below 1 yr of age were calculated to be 1.1 and 2.3 as the mean and 90th percentile, respectively (Attachment 17), according to methodology of ENVIRON for Frutafit® (GRN 118, FDA, 2002). Based upon these estimated exposure values and the Japanese infant survey (Yamamoto and Yonekubo, 1993), the Panel believes the food uses and at the levels specified herein are GRAS.

### **6.2.3** Safety of Inufib™

6.2.3.1 Common knowledge elements of the GRAS determination

The first common knowledge element for a GRAS determination is that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing published, peer-reviewed scientific journals for the safety assessment. The majority of the studies reviewed in this safety assessment have been published in the scientific literature. The common use of agave inulin and its associated components in food on a global basis and the associated absence of harm are based upon published information of all types including clinical studies, which support the safety assessment, which have also been published in the scientific literature.

Major critical reviews of well known experts in the field of food toxicology (e.g., Roberfroid and Delzenne, 1998; Carabin and Flamm, 1999) published comprehensive and critical reviews of the available data and information---both published and unpublished--- and unanimously concluded that under the conditions of intended use in foods, inulin-type fructan is GRAS based on scientific studies that the authors reviewed. These reviews clearly note that there is no evidence of acute, chronic reproductive or developmental toxicity, carcinogenicity or genotoxicity in tests at dose levels considerably higher than anticipated human exposure. Interestingly, the rate limiting step with agave inulin-type fructans is its affect on the gastrointestinal tract.

Clinical studies on the intake and tolerance of inulin-type fructans also show signs of gastrointestinal intolerance are observed with intakes above 20–30 grams as described in Section 5.6.2. Roberfroid and Delzenne (1998) indicate that fructans are better tolerated when given with solid food and when given in divided doses throughout the day. Carabin and Flamm (1999) summarized 20 published studies analyzing the gastrointestinal symptoms and tolerance of inulin-type fructans and concluded that they are safe for human consumption under intended conditions of use as a dietary fiber, and that up to 20 g/day of inulin and/or oligofructose is well tolerated. More recently, Holscher et al. (2014) demonstrated that healthy adults who consumed daily doses of 7.5 g agave inulin fiber in a single serving (highest dose given) for three consecutive 21-day periods did not report any serious gastrointestinal symptoms, and López-Velázquez et al. (2013) reported that infants who consumed formula containing 0.5 g / 100 mL *Agave tequilana* fructans for a six-month period did not experience a significant increase in GI symptoms such as colic, abdominal distention, flatulence, and regurgitation.

The second common knowledge element for a GRAS determination requires establishing that a consensus exists among qualified scientists about the safety of the substance with its intended use. As previously noted, in 1998 (Roberfroid and Delzenne, 1998) and in 1999 (Carabin and Flamm, 1999) literature reviews and analyses of all available data (published and unpublished) were published in peer-reviewed publications both of which conclude that agave inulin-type fructans are GRAS.

Major points regarding the safety of agave inulin-type fructans made by these authors include:

- Fructans are closely-related linear or branched fructose (oligo) polymers, which are either beta-2,1-linked inulins or beta-2,6-linked levans. Inulin is defined as a polydisperse

carbohydrate material consisting mainly, if not exclusively, of beta-(2-1) fructosyl-fructose links.

- This class of polymers (inulin-type fructans) is present in significant amounts in miscellaneous edible fruits and vegetables with the average daily consumption having been estimated to be 1-4 g in the United States and 3-11 g in Europe.
- Studies have demonstrated that inulin-type fructans, when administered in the diet at high levels, do not result in mortality, morbidity, target organ toxicity, reproductive or developmental toxicity, or carcinogenicity.
- Inulin-type fructans have been classified as nondigestible oligosaccharides; there is no evidence that they are absorbed to any significant extent.
- Both *in vitro* and *in vivo* studies on the fermentation of inulin type fructans demonstrate that they are metabolized by anaerobic bacteria that are normal constituents of the colonic microbiota.
- Inulin-type fructans are thus bifidogenic and they are classified as "prebiotics," i.e., "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon and thus improves host health."
- Inulin-type fructans are likely to affect positively calcium absorption and calcium balance, including in humans.

Further consensus evidence is provided in a committee of experts convened by BENEO-Orafti (Belgium) in 1992 to conduct a GRAS self affirmation (Kolbey et al, 1992). While the report was not published in a peer-reviewed journal, the committee members were imminently qualified and their findings are still referred to in many published articles and regulatory documents on inulin-type fructans (i.e., Coussement, 1999, GRN-44 [FDA, 2000a]; GRN-118 [FDA 2003]). The committee was composed of Albert C. Kolbye, Herbert Blumenthal, Barbara A. Bowman, John H. Byrne, C. Jelleff Carr, John C. Kirschman, Marcel B. Roberfroid and Morris A. Weinberger. This expert committee found the following:

- Inulin and oligofructose are not hydrolysed in the stomach or small intestine, but are fermented completely into harmless metabolites in the colon, where they are specific substrates for the growth of Bifidobacteria.
- Available animal toxicity studies are consistently free of any suggestions of adverse effects to be expected from such proposed levels of use in foods.
- Inulin and oligofructose are dietary fibers by definition and, by their nutritional properties; intake is self-limiting because of a gaseous response in the colon that prevents over-usage.
- The safety of inulin and oligofructose is based on the long human experience of consuming inulin containing foods as well as evaluation of available scientific evidence relating to inulin and its hydrolysis products. Further, since inulin and oligofructose have been natural components of many foods consumed safely by humans over millennia, there is no reason to suspect a significant risk to the public health when used in foods as intended by the notifier.
- These food substances are generally recognized as safe, both by a long-established history of use in foods and by the opinion of experts qualified by scientific training and experience in food safety after a thorough review of the available scientific evidence.

Additional consensus elements include the reviews of the global expert bodies such as the EU and the FDA, as well as the authorities in Australia, New Zealand, Canada, and Japan.

In the United States, chicory inulin was determined to be GRAS without questions by FDA (GRN 118, FDA 2002) and fructooligosaccharide (which is a shorter chain length fructan produced by enzymatic synthesis from sucrose), was determined to be GRAS without questions by FDA (GRN 44, FDA, 2000). The FDA had no questions about either of these GRAS assessments (FDA, 2000b; FDA, 2003).

Inulin is legally classified as food or food ingredient in most countries including all European Union (EU) countries, Australia, Canada, and Japan (Franck, 2002). As a food or food ingredient, inulin can be used without specific limitations as ingredients in foods and drinks. The EU Standing Committee meeting of June 1995 confirmed oligofructose as a food ingredient (EC, 1995). Inulin is classified as a food ingredient and not a food additive according to the European Directive 95/002 on Food Additives (EC, 1995), and all the EU countries list inulin as having food ingredient status.

In March, 2006, Canada's Health Products and Food Branch approved the classification of inulin as a dietary fiber in Canada. The Canadian Food Inspection Agency (2011) lists "chicory root inulin" as a traditional fiber source. "Inulin from Jerusalem artichoke tuber" is similarly classified as a traditional fiber source (Canadian Food Inspection Agency, 2011).

Food manufacturers have added inulin-derived substances to the general food supply in Australia and New Zealand since the mid 1990s. Since 2001, inulin has appeared in a wide range of foods and is predominantly labeled as dietary fiber. The FSANZ (Food Standards of Australia and New Zealand) Food Standards Assessment Report dated July 16, 2008 declared that "There is a history of safe use of inulin-derived substances in food in Australia and New Zealand, so food manufacturers do not need express permission to add these substances to the general food supply (FSANZ, 2008). However, it should be noted that while fructooligosaccharides are permitted in infant formula in the EU and in the US, they not similarly permitted in the New Zealand or Australia at the date of this GRAS assessment.

6.2.3.2 Panel findings

The Expert Panel has reviewed the substantial body of data in the published literature on agave inulin and inulin-related fructans such as oligofructose and fructooligosaccharide in animal and clinical studies, the major comprehensive critical reviews on inulin and inulin-related fructans, the international regulatory summaries, the previous GRAS submissions on inulin and the fructooligosaccharide and has considered the FDA no questions responses. In addition the Panel conducted a comprehensive literature and databank search and conducted a critical review of the Inufib™ production process and has concluded that IIDEA's Inufib™ at the usage levels described herein is generally recognized as safe (GRAS) in foods.

The following is a summary of the critical elements that were taken into consideration in the safety evaluation of inulin, oligofructose and fructooligosaccharides.

- **Chemical structure studies** show that agave fructan consists of branched inulin-levan type fructans, composed of fructose units joined by β(2→1) and β(2→6) glycosidic linkages, and 1,6 fructofuranose branches, with one glucose moiety per molecule consistent with a terminal position. The average degree of polymerization of agave inulin from *Agave tequilana* plants ranges from approximately 14 to 18, and is centered around 16, with some variation based on the age of the plant and the region of cultivation. The degree of polymerization for agave inulin is consistent with that of other inulins consumed by humans, including native chicory root inulin, which has an average DP of 10-20. The types of linkages in these fructans vary quantitatively but are qualitatively similar.
- **Other constituents** include 10 fatty acids and 32 terpenes which contribute to the characteristic flavors of the alcoholic products of agave. The total fatty acid content was 985 μg/g; or approximately 0.1% where predominant fatty acids were linoleic acid (448 μg/g) and palmitic acid (~257 μg/g) followed by oleic acid and linolenic acid (~ 100 μg/g each). The overall terpene concentration was extremely low with linalool being the primary terpene component. The estimated potential concentration of linalool in dried agave inulin, based on its measurement in tequila, is ~0.4 mg/kg (~0.4 ppm). Saponins have not been detected at levels as low as 7 ppb.
- **Metabolism and gastrointestinal tract studies** show that agave inulin is resistant to hydrolysis by human digestive enzymes and will pass largely intact to the colon where it is subject to fermentation by colonic microflora. *In vitro* assessment of the pontential for prebiotic effects of fructans showed an efficient stimulation of growth of Bifidobacteria and Lactobacilli. *In vivo* studies showed that fermentable fructans are able to promote the production of satietogenic/incretin peptides also revealing prebiotic effects.
- **Animal studies** show fructooligosaccharides display a low order of toxicity in all animal testing yielding a rat oral $LD_{50}$ value >9 g/kg bw; a 6 week rat study with diets enriched with 5 or 10% fructooligosaccharides showed no toxicity compared with existing sugars commonly used in the food supply; reproductive and developmental studies on fructooligosaccharides in rats showed no significant effects on the course of pregnancy or on the development of fetuses and newborns; a rat 4-week toxicity study on carboxymethyl inulin by the gavage route showed no treatment-related effects in body weight, food consumption, mortality, hematology, clinical blood chemistry, organ weights or gross or microscopic pathology up to the highest dose of 1000 mg/kg-day; A guinea pig Magnusson–Kligman maximization test showed no evidence of dermal sensitization with carboxymethyl inulin; 6 week studies examining health protection in mice feeding inulin or oligofructose to mice prior to enteric challenges and systemic bacterial infections resulted in an increased host resistance to the challenges concluding that gastrointestinal tract bacteria remain responsive to long-term feeding of fructan prebiotics; a study of the effect of chicory-derived fructans on azoxymethane-induced colon cancer in male F344 rats showed a decrease in azoxymethane-induced carcinogenesis; 14 week study of fructans extracted from onion revealed prebiotic effects as evidenced by decreased pH, increased butyrate and propionate production and an increase in the cecal microbiota enzyme activities, β-glucosidase and β-glucuronidase.

- **Genotoxicity and mutagenicity** studies on fructooligosaccharides, carboxymethyl inulin, and agave inulin have shown no *in vitro* mutagenesis or clastogenesis.
- **Carcinogenicity** was not evident after a 2 year rat carcinogenicity study with fructooligosaccharides diets at concentrations of 0, 8000, 20,000, and 50,000 ppm, which revealed no significant dose-related effects on body weight, food consumption, survival, growth, hematology, blood chemistry, or organ weights, nor did the treatment affect the incidence of neoplasms.
- **Clinical studies** also show tolerance to inulin-type fructans in historical and contemporary diets and in clinical studies employing bolus, short-term, and long-term exposures. By consensus, inulin-type fructans have been classified as "nondigestible" oligosaccharides, which positively affect the composition and metabolic activity of the intestinal microflora of humans. Consensus was also reached on the human evidence for the stool regulating effect of inulin-type fructans and the increase in calcium and magnesium absorption with moderate supplement intake. Studies have shown that fructans cause significant decreases in total cholesterol, blood glucose level, triglycerides and low density lipoproteins; All nondigestible carbohydrates including inulin-type fructans, may cause intestinal discomfort and possible laxative action that is dose-related as a result of fermentation in the large bowel; however, experts concluded that increased exposure to inulin and oligofructose is likely to be of negligible biological significance at the 90th percentile.
- **The production process** for Inufib™ from premium agave involves the mechanical extraction of the juice from the pine (piñas) of the blue Agave without the use of solvents or other chemicals. The production process has numerous certifications, and analyses reveal there are no biocides present and it meets all microbiological and heavy metal standards.
- Agave inulin and inulin-related fructans have a **long history of safe use.** Human remains dating back at least 10,000 years show early uses of agave for food and fiber. It was exported to Europe by 1520, and was mentioned as a food of Aztecs and natives in the Florentine Codex of 1580. Fructan-containing products derived from many of these plants are commercially available and sold online and in health food stores in the United States In 1992 Kolbye et al (1992) performed a GRAS self affirmation for BENEO-Orafti. Two GRAS affirmations have been submitted to FDA (FDA, 2000a, GRN 44; FDA, 2002, GRN 118) with no question responses from the FDA (FDA, 2000b; FDA, 2003). Inulin is legally classified as food or food ingredient in most countries including all EU countries, Australia, Canada, and Japan (Franck, 2002). The EU Standing Committee meeting of June 1995 confirmed oligofructose as a food ingredient (EC, 1995). In March, 2006, Canada's Health Products and Food Branch approved the classification of inulin as a dietary fiber in Canada. The FSANZ (Food Standards of Australia and New Zealand) Food Standards Assessment Report dated July 16, 2008 declared that "There is a history of safe use of inulin-derived substances in food in Australia and New Zealand, so food manufacturers do not need express permission to add these substances to the general food supply (FSANZ, 2008).

The Expert Panel concludes that consensus exists regarding the safety of the intended human food uses of inulin-realted fructans based upon the peer-reviewed literature including individual studies and critical general reviews; previous GRAS submissions by the GTC Company on

fructooligosaccharides and Imperial-Sensus, LLC. on Frutafit® (inulin derived from chicory root) both of which received no questions agency responses, the various in-depth reviews by experts found in published and unpublished sources, the numerous global regulatory agency approvals for use in food and beverages as well as regulatory bodies in the US, Canada, Mexico, Japan, Australia and New Zealand all of which have concluded that inulin is safe for use in food.

## 7.0 CONCLUSIONS[11]

The Expert Panel has carefully reviewed and evaluated this publicly available information summarized in this document and the production data available from IIDEA as well as consideration of the potential human exposure to this compound, and has made the following determination:

**The Expert Panel concludes that use of Inufib™ in foods, when produced in compliance with Good Manufacturing Practices requirements and which meets the specifications established by IIDEA as presented in this document, is generally recognized as safe at dietary levels expected from the proposed uses.**

This declaration is made in accordance with FDA's standard for agave inulin safety, i.e., reasonable certainty of no harm under the intended conditions of use.

Richard C. Kraska, Ph.D., DABT
Chair

[11] The detailed educational and professional credentials for two the individuals serving on the Expert Panel can be found on the GRAS Associates website at www.gras-associates.com . Drs. Kraska and McQuate worked on GRAS and food additive safety issues within FDA's GRAS Review Branch earlier in their careers and subsequently continued working within this area in the private sector. Dr. Kapp's curriculum vitae can be accessed at http://www.biotox.net. All three panelists have extensive technical backgrounds in the evaluation of food ingredient safety. Each individual has previously served on multiple GRAS Expert Panels. Dr. Kraska served as Chair of the Panel.

Robert S. McQuate, Ph.D. Robert W. Kapp, Jr., Ph.D., Fellow ATS

**DATE: November 8**, 2011

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updated Jan 15 2015

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# 9.0 APPENDIX

## 9.1 Scientific Literature on Chemical Identity of Agave Inulin and Raw Material

### 9.1.1 Carbohydrate Composition and Degree of Polymerization

Water soluble carbohydrates from the heads of *Agave tequilana* Weber var. azul were evaluated by Waleckx et al. 2008. Pulp produced from the transversal cutting of six mature A. tequilana heads were placed in a mixer with distilled water at 80°C and agitated for 5 minutes to extract a suspension of water soluble carbohydrate, which was filtered. The water soluble carbohydrate content of the agave heads was 28.3 g/100 g (fresh weight) ± 0.1% and 86.7 g/100 g (dry weight) ± 1.3%. Based on high performance liquid chromatography analysis, 93.4% of the carbohydrates consisted of fructans having DP ≥ 3.2% were free disaccharides, 0.8% free glucose (0.8%), and 3.8% free fructose. The average DP of fructans in the extract was 13.6 ± 1.3.

The water soluble carbohydrate composition and fructan structures of *Agave tequilana* plants grown in different regions of Mexico were investigated by Mancilla-Margalli and Lopez (2006). Carbohydrate content varied with climatic conditions. Fructan fractions from *A. tequilana* grown in Jalisco consisted mainly of molecules with a high degree of polymerization, and an estimated average DP of 18; fructooligosaccharides were absent. Monosaccharides were exclusively glucose and fructose. Both internal (4 – 7%) and terminal (1 – 8%) glucose moieties were present in fructans from Agave species, in proportions dependent on the region of cultivation.
Arrizon et al. (2010) further analyzed the carbohydrate composition and fructan structures of *Agave tequilana* plants of different ages, by HPLC, HPAEC-PAD, MALDI-TOF-MS and GC-MS confirming the presence of both terminal and internal glucose moieties. The degree of branching and the ratio of terminal to internal glucose moieties were higher in 4 year-old plants, compared with either 2 year- or 6.5 year-old plants. High performance liquid chromatographic analysis indicated that 2-year old plants contained the highest levels of free monosaccharide (fructose and glucose) and low molecular weight fructans (DP 3 – 6) with total fructan content comprising 69% of the total carbohydrate content. Fructan content of the 4 and 6.5 year old plants was 97% while free fructose, glucose and sucrose each accounted for < 1% of the carbohydrate content. The degree of fructan polymerization was greatest in the 4 year-old plants with the DP ranging from 3 - 30, and decreased to DP 4 - 24 in 6.5 year-old plants.

Low molecular weight fructans (D.P. range 3-5) account for approximately 9% of the total (Toriz et al, 2007). Other than fructose and glucose, no other monosaccharides were identified in the analysis of *Agave tequilana* Weber var. azul. (Mancilla-Margalli and Lopez, 2002; Lopez et al. 2003; Ávila-Fernández et al., 2009).

Thus, the average degree of polymerization of agave inulin from *Agave tequilana* plants ranges from approximately 14 to 18, and is centered around 16, with some variation based on the age of the plant and the region of cultivation. The degree of polymerization for agave inulin is consistent with that of other inulins consumed by humans, including native chicory root inulin, which has an average DP of 10-20 (Roberfroid and Delzenne, 1998).

### **9.1.2** Molecular Structure and Chain Length Distribution of Agave Inulin

Fructans are polydisperse carbohydrate molecules which vary with respect to size and linkage type. Agave inulin consists of a mixture of branched fructan polymers containing β(2→1) and β(2→6) linkages, and 1,6-linked fructofuranose branches with a single glucose moiety per molecule in either an internal or terminal position (Lopez et al. 2003; Mancilla-Margalli and Lopez, 2006; Toriz et al. 2007; Arrizon et al. 2010). Characterization of linkages was accomplished with GC–MS, $^{13}$C NMR, $^{1}$H NMR and MALDI-TOF-MS. The chemical structure of agave inulin was published by Toriz et al. (2007- attachment a1) and Lopez et al. (2003 – Attachment 18).

Reductive cleavage, size exclusion chromatography/light scattering and $^{13}$C NMR were used to characterize the composition and chain length distribution of agave fructans from the blue agave plant (*A. tequiliana* Weber var. *azul*) (Toriz, et al. 2007). The range of chain lengths was from DP 3 to DP 60, and the mean chain length was DP of 16. Agave fructan consists of branched inulin-levan type fructans, composed of fructose units joined by β(2→1) and β(2→ 6) glycosidic linkages, and 1,6 fructofuranose branches, with one glucose moiety per molecule consistent with

a terminal position (Toriz et al., 2007) The mean number of fructose units (i.e., degree of polymerization) in agave inulin is 16, with a range of 3 to 60 fructose units, where

$$D.P. = \sum(\text{fructose} + \text{glucose})/\text{glucose}$$

and the number average molecular weight is $M_n$ = 2690 g/mol. Low molecular weight fructans (D.P. range 3-5) account for approximately 9% of the total, and the molecular weight distribution of agave fructans ranges from 3 to 60 fructose units (Toriz et al., 2007).

The structural characterization of Agave fructans by Toriz et al. (2007) is similar to the analysis of fructans extracted from 8 year old *Agave tequilana* Weber var. azul plants (Lopez et al. 2003) in which the linkage types present were determined by permethylation followed by reductive cleavage, acetylation, and finally gas chromatography-mass spectrometry (GC-MS) analysis. Analysis of the degree of polymerization (DP) estimated by $^{1}H$ NMR integration and $^{13}C$ NMR showed that *A. tequilana* fructan is comprised of at least 16 residues, with the glucose/fructose ratio of at least 1:15. Matrix-assisted laser desorption time-of-flight mass spectrometry (MALDITOF-MS) confirmed a molecular weight distribution of 527-4739 Da, and a DP ranging from 3 to 29 units. The 8-year old plant included a Fru*f* residue linked in a β(2→ 6) form with an internal glucose (Lopez et al. 2003).

**9.1.3** Other Constituents from the Tissue of the *Agave tequilana* Piñas

Pena-Alvarez et al., (2004) analyzed *Agave tequilana* Weber var. azul, *A. salmiana*, and *A. angustifolia* for terpenes and fatty acid content which contribute to the characteristic flavors of the alcoholic products of agave. Terpene content was determined on 160 g samples of fresh or frozen agave piñas tissue using steam distillation extraction–solid-phase microextraction coupled to GC–MS; identification was accomplished by comparison of retention times with those of standards, Kovats Index and the NIST mass spectrometry library. Fatty acids as their ethyl esters were determined on 50 g samples of agave piñas tissue by Bligh–Dyer extraction–derivatization coupled with gas chromatography, identified with external standards and confirmed by mass spectra. In all the Agave species tested, ten fatty acids were identified. With the quantities found in the *Agave tequilana* samples presented in parentheses, the predominant fatty acids were linoleic acid (448 μg/g) and palmitic acid (~257 μg/g) followed by oleic acid and linolenic acid (~ 100 μg/g each). Others included lauric acid, myristic acid, pentadecylic acid, palmitoleic acid, margaric acid and stearic acid, present at concentrations ranging from ~5 to 30 μg/g. Total fatty acid content in *Agave tequilana* was 985 μg/g; or approximately 0.1%. The authors considered it likely that some of the fatty acids found in tequila came from the *Agave* raw material and did not undergo any modification during the cooking, fermentation and distillation process. Terpenes were difficult to identify due to their low concentrations in the plants and poor resolution by gas chromatography. Thirty-two terpenes were detected in *A. tequilana*, but they were not quantified. The main terpene in the three Agave plants was linalool (Pena-Alvarez et al., 2004).

### **9.1.4** Chemical Constituents of Other Food Products Derived from Agave Piñas

Linalool is also present in tequila, which is produced by the hydrolysis of the fructans obtained from the piñas of *A. tequilana*. Since it was detected in the *A. tequilana* plant (Pena-Alvarez et al, 2004), it is likely that some if not all of the linalool content in tequila originates from the raw material, although some may be introduced through the production process. In tequila production, the agave piñas (agave cores) are cooked, crushed to extract the juice, and then fermented to produce alcohol. The raw material undergoes numerous chemical and biochemical reactions leading to a distilled tequila product containing approximately 200 different compounds. The composition of the product is dependent on plant maturity, cooking, yeast fermentation and distillation processes. Some of the compounds that impart aroma and flavor characteristics are alcohols, fatty acids, esters, aldehydes, terpenes, phenols, lactones, sulfur compounds. Ávila-Fernández et al., (2009) measured the concentrations noncarbohydrate components of tequila by GC-MS analysis; notably of terpenoids including linalool. Tequila was produced by the traditional process involving exclusively thermal hydrolysis of fructans prior to fermentation. The combined concentration of linalool and its oxides was 0.5 mg/L tequila. Other constituents that were quantified included free fatty acids and fatty acid ethyl esters (100-150 mg/L); alcohols and esters (200- 250 mg/L); cyclic oxygenated compounds (20-50 mg/L); and terpenoids (1-3 mg/L).

Most ripe agave heads average 50-60 kg and can yield 7.1 to 8.5 liters of tequila[12]. If all of the linalool in tequila originates in the plant, one ripe agave head can be estimated to contain (0.5 mg linalool/L x 7.1 – 8.5 L) or 3.6 – 4.3 mg linalool/head. This value can be used as the basis for estimating the quantity of linalool in the pure dried inulin produced from agave piñas. Assuming a ratio of raw agave to pure dried inulin of 5.33:1[13] substances in the raw agave can theoretically be concentrated 5.33-fold during the production of pure dried inulin. If the concentration of linalool in agave is 4 mg/55 kg, the dried inulin might be expected to contain ~21 mg/55 kg or 0.38 mg/kg.

Agave inulin does not contain Maillard compounds. Maillard compounds are generated from thermal processing of *A. tequilana* Weber var. *azul* during tequila production (Mancilla-Margalli and Lopez, 2002). After cooking the plant stems at 100°C for 4 to 32 hours, the most abundant Maillard compounds generated were the furans, methyl-2-furoate and 5-(hydroxymethyl) furfural, and the pyran, 2,3-dihydroxy-3,5-dihydro-6-methyl-4(H)-pyran-4-one. Also present was furfural, shown to be formed from the thermal processing of other fructan containing crops including wheat, rye, barley, and chicory (Frank, and Hofmann 2000). These Maillard products impart sweet notes contributing to the flavor of tequila. Since the production of agave inulin does not involve the thermal hydrolysis of fructans, agave inulin does not contain Maillard compounds.

Hydrolyzed agave juice from *Agave salmiana* Otto ex Salm-Dick was analyzed for sugar content by high performance liquid chromatography with refractive index detection. The only sugars identified were xylose, fructose, glucose, sucrose, and maltose *(*Michel-Cuello et al., 2008).
Agave syrup (also known as blue agave syrup and agave nectar) is also produced from the juice of agave piñas that has been heated or treated enzymatically to hydrolyze the fructans to fructose

[12] "In search of the blue agave" (http://www.ianchadwick.com/tequila/production.htm)

[13] The article "Inulin answers agave surfeit problem" indicates that 800 tons of raw agave yield 150 tons of pure, dried inulin. http://www.nutraingredients-usa.com/Industry/Inulin-answers-agave-surfeit-problem

monomers, and subsequently concentrated to syrup (Mancilla and Lopez, 2002). The taste and consistency of agave nectar are similar to corn syrup owing to the high fructose content. Agave syrup was among the plant syrups and juices that were analyzed for sugar content, amino acid content and 5-(hydroxymethyl)furfural concentration, to assess amino acid racemization through formation of fructose-amino acids (Amadori compounds) formed during the Maillard reaction. Sucrose, D-glucose, and D-fructose were determined using an enzymatic assay and amino acids by enantioselective gas chromatography-mass spectrometry. 5-(Hydroxymethyl)furfural served as an indicator for heat treatment and progress of the Maillard reaction and was assayed colorimetrically after derivatization with barbituric acid/p-toluidine. D-Ala was detected in all plant products and amounted to 13.5% D-Ala (relative to L-Ala + D-Ala) in agave syrup; similar D-Ala was found in pomegranate, palm and grape syrups, while mean D-Ala content in Canadian maple syrups ranged from 33-34%. No other D-amino acids were also detected in Agave or grape concentrate (Arrope); whereas several other D-amino acids were found in the other syrups and juices. The quantities of glucose and fructose in agave syrup were 19.9 and 55.6%, respectively. Sucrose was not detected. 5-(Hydroxymethyl)furfural concentration ranged from 7 mg/100 g in Agave syrup to 14.5 g/100 g in Arrope (Pätzold and Brückner, 2005).

García-Aguirre et al. (2009) investigated methods to optimize the production of fructose-rich syrups via enzymatic hydrolysis of agave fructo-oligosaccharides. The substrate was fructo-oligosaccharides in agave juice obtained from fresh “heads” or “pines” (plants without leaves) of *Agave tequilana* Weber var. *azul*. The source of the enzyme having inulinase activity was the yeast Kluyveromyces marxianus, endogenous to Aguamiel obtained from traditional rural producers of pulque of Guanajuato state in Mexico. Conditions were optimized for maximum inulinase synthesis and hydrolysis of agave fructo-oligosaccharides. HPLC analysis of the fructose-rich syrups obtained at these optimal conditions showed an average composition of 95% of fructose and 5% of glucose and the absence of sucrose. The analysis also revealed that the syrups are free of contaminants such as hydroxymethylfurfural, which may be present in products obtained by thermal or acid hydrolysis. Since thermal and acid hydrolytic processes are not relevant to the production of agave inulin, hydroxymethylfurfural and related contaminants are not present.

The total antioxidant content of Agave nectar as compared with other natural sweeteners and refined sugar was determined using the ferric-reducing ability of plasma (FRAP) assay. Major brands of sweeteners, refined white sugar and corn syrup were sampled from retail outlets in the United States. Five agave nectar products were analyzed and found to contain minimal antioxidant capacity, comparable to refined white sugar or corn syrup. The two brands of blue Agave nectar analyzed contained 0.034 mmol FRAP/100 g (Molina Real) and 0.143 mmol FRAP/100 g (Live Superfoods). The antioxidant capacity of the other three Agave nectar products brands analyzed: “light”, “raw”, and “amber” Agave nectars (Madhava), was <0.03 mmol FRAP/100 g (Phillips et al., 2009).The main sugars identified in aguamiel are glucose, sucrose, fructose and several pentoses (Sanchez-Marroquin and Hope, 1953, as cited by Tovar et a. 2008).

Aguamiel from Agave mapisaga plants was analyzed to determine its chemical composition by Ortiz-Basurto et al. (2008). It contained 11.5 wt % of dry matter, which consisted mainly of sugars (75 wt %). Fructose (32 wt %) and glucose (26 wt %) were the principle components followed by fructo-oligosaccharides which accounted for 10 wt % and sucrose at 9 wt %. Protein accounted for

3 wt%. Free amino acids constituted 0.3 wt % and included most essential amino acids and γ-aminobutyric acid, glycine, asparagine/aspartate and glutamine/glutamate.

Agave derived pulque and aguamiel were analyzed for phytase activity and ascorbic acid, iron, zinc, calcium, magnesium and selenium contents. Pulque and aguamiel samples from several producers located in the states of Tlaxcala, Puebla and Hidalgo were pooled and stored at 4 °C. Iron, zinc, calcium and magnesium in the samples were quantified by flame atomic absorption spectroscopy according to the AOAC (method 985.35) and selenium was determined by hydride generation. Ascorbic acid was determined in samples of liquid pulque and aguamiel, according to the AOAC (method 967.21). The ascorbic acid content of two of the liquid samples of pulque from different dates was 2.66±0.12 mg/100 ml, and was negligible in a third sample. Ascorbic acid content in two different samples of aguamiel was 2.01± 0.10 mg/100 ml, indicating that ascorbic acid is an endogenous metabolite of the Agave. The concentrations of calcium, magnesium, selenium and iron in fresh pulque were 20.4, 16.4, 1.3, and 0.03 μg/100 g, respectively; and in aguamiel were 25.8, 13.8, 1.3, and 0.03 μg/100 g, respectively. Zinc was not detected in pulque or aquamiel. Phytase activity was also found the pulque and aquamiel samples and the authors proposed that phytase from live bacteria in pulque dephosphorylates phytate in the gastrointestinal tract of humans, improving the bioavailability of iron and zinc (Tovar et al. 2008). A typical 0.5 L serving of pulque contains 30 mg of ascorbic acid, 0.1 mg of thiamin, 0.1 mg of riboflavin, and 3.5 mg of iron, and contains approximately 4%–6% ethanol (Kuhnlein, 2004).

### **9.1.5** Chemical Constituents of Agave Whole Plants, Roots, Leaves and Fruits

Agave plants typically have long spine-like leaves with needles along the edges. Leaves can produce a liquid that can be irritating when it comes in contact with human skin. Workers in tequila distilleries and on agave plantations may develop an irritant contact dermatitis, which was determined by Salinas et al. (2001) to be attributable to the presence of sharp, needle-like calcium oxalate crystals, known as raphides, in the plant. Salinas et al. (2001) isolated and purified calcium oxalate crystals from the leaves of *A. tequilana*. The crystals were characterized as 30–500 μm in length, sharpened at both ends. One drop of juice pressed from the leaves contained 100 – 150 of the needle-like crystals.

The agave genus is an important source of steroidal sapogenins, among them, hecogenin, tigogenin and diosgenin, used for the production of contraceptives, corticosteroids, and steroidal diuretics, among other therapeutic applications (Crabbe, 1979; Bedour et al., 1979; Garcia, 2000; Narvaez-Zapata and Sanchez-Teyer, 2009; Ruvalcaba-Ruiz and Rodriquez-Garay, 2002). Saponins are potentially toxic, but are present in many other edible plants including lettuce, onions, oats, spinach, most beans and legumes, paprika, and alfalfa. Agave saponins have been investigated for their antimicrobial and antifungal properties, as well as anti-inflammatory and immune-stimulating properties. Saponins are characterized structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. The aglycone is referred to as the sapogenin and steroid saponins are called saraponins. The combination of the nonpolar sapogenin and the water soluble side chain are the basis for the foaming properties of saponins and their use for soaps (Cornell, 2009).

Saponins are typically isolated from Agave leaves or roots by methanolic or organic solvent extraction. Steroidal sapogenins have been isolated from leaves of *Agave lecheguilla, A. sisalana, A. lophantha,* and *A. parasana* and *A. utahensis,* and *A. Americana* (Bedour et al., 1979; Blunden et al. 1974)*;* from the flowers of Agave salmiana (Maguey) (Sotelo et al. 2007); from the fruits of *A. cantala* (Uniyal et al. 1991); from the roots and seeds of *A. lechuguilla* and from the whole plants of *A. utahensis. Agave lechuguilla* contains a saponin in the rootstocks and leaves, which are used locally as soap substitutes and in shampoo mixtures. Saponins that have been identified in the various species of Agave are presented in the Appendix Table A-1. Chen et al. (2009) isolated three known flavones: 5,7-dihydroxyflavanone, kaempferol 3- rutinoside-4-glucoside, and kaempferol 3-(2G rhamnosylrutinoside); and seven homoisoflavonoids: 7-*O*-methyleucomol, 3-deoxysappanone, (±)-3,9-dihydroeucomin, dihydro-bonducellin, 7-hydroxy-3-(4-hydroxybenzyl) chromane, 5,7- dihydroxy-3- (4-hydroxy-benzyl)-4-chromanone and 5,7-dihydroxy-3-(3-hydroxy-4-methoxybenzyl)-4-chromanone), from methanolic extracts of the leaves of A. sisalana (Debnath et al. 2010).

Hecogenin and tigogenin are the two most abundant sapogenins in the mature leaves of *A. sisalana* (Cripps and Blunder, 1978). Their concentrations in extracts of the leaf and leaf juice were determined using a gas-liquid chromatographic method of the acetylated derivatives. Hecogenin content was up to approximately 1% of the leaf extract and 0.14% in the leaf juice. The corresponding tigogenin contents were approximately one tenth of the hecogenin content.

Saponins and flavones have not been detected in stem extracts of *Agave tequilina* plant, nor have they been reported in the inulin fraction of *Agave tequilana*. There is no evidence of the presence of toxic saponins in agave inulin, either from compositional analysis of agave pin a extracts or the long history of use of Agave stems for food and spirits.

### **9.1.6** Classification of Agave Inulin among Edible Plant Fructans

Five major types of fructans have been identified in nature according to the type of β-fructofuranosyl linkages and position of glucose in the structure (Vijn et al. 1997): (i) linear inulin with β(2→1) -fructofuranosyl linkages and a terminal glucose, commonly found in chicory and other plants in the Asteraceae family, (ii) neoseries inulin, which contains an internal glucose moiety between two fructofuranosyl units extended by β(2→1) linkages, characterized in onion (*Allium cepa)* and asparagus *(Asparagus officinalis)*, (iii) levans with linear β(2→ 6) linkages with a terminal glucose, found in grasses like *Phleum pratense*, (iv) Neoseries levans, formed by β(2→1)- and β(2→ 6)-linked fructofuranosyl units on either end of a central glucose molecule, which has been reported in oat (*Avena sativa)*; alternatively they are composed of two linear β(2→ 6)-linked fructosyl chains, having an internal glucose moiety, and (v) Mixed fructans containing β(2→1) and β(2→ 6) linkages; generally the fructans of this group are branched like those found in wheat (*Triticum aestivum)* and agave. The glucose moiety may be terminal (graminans) or internal (agavins) (Mancilla-Margalli and Lopez, 2006; Waleckx et al., 2008).
According to the above system for classification of fructans, agave inulin belongs to the "mixed fructan" group based on the two linkage types and chain branching. Agave fructans were further categorized as graminans, (mixed fructans containing branched β(2→1) and β(2→6) linkages and terminal glucose moieties), and agavins (branched neo-fructans, characterized by internal α-D-glucopyranose) (Mancilla-Margalli and Lopez, 2006).

The degree of polymerization as well as the types of linkage which predominate in the fructan molecules depends on the type of fructan biosynthetic enzymes present in the plant. Phylogenetic analysis based on the presence of two such enzymes - vacuolar invertases and fructosyltransferases; places *Agave tequilana* within the Asparagales branch, closely related to Allium cepa (common onion) and Asparagus officinalis (asparagus) (Van den Ende, et al., 2011).

Analysis of the structural diversity of fructan-rich plants indicates quantitative more than qualitative differences among the species. For example, plants from the Asterales order such as chicory and dahlia contain predominantly linear polysaccharides with β(2→1) linkages (i.e., "linear inulins" group), but branched fructans and β(2→6) linkages are also present (Van Loo et al., 1995; Roberfroid and Delzenne, 1998; Mancilla-Margalli and Lopez, 2006). Likewise, a small fraction of the total agave fructan contains fructans from the linear inulin series (Waleckx et al. 2008). The term "inulin" for fructans of chain length > 10 has been applied generically to the various fructans owing to the fact that they were first isolated from *Inula helenium* (Toriz et al. 2007).

### 9.2 Effects of Non-Carbohydrate Constituents of the Agave Plant

Moderate pulque consumption in the central highlands of Mexico as a part of the maternal diet is associated with better infant birth size and growth than non-use of pulque (Kuhnlein, 2004). It is approximately 5% ethanol, and a 0.5 L serving provides significant nutrients and minerals, including ascorbic acid, thiamin, riboflavin and iron.

Agave plants typically have long spine-like leaves with needles along the edges. Several known irritants are present in sap of agave leaves, including calcium oxalate raphides, acrid oils, and saponins. Irritant contact dermatitis was relatively common among workers in tequila distilleries and on agave plantations. During their investigation of these workers, Salinas et al. (2001) isolated and purified calcium oxalate crystals from the leaves of *A. tequilana*. The crystals were characterized as 30–500 μm in length, sharpened at both ends, and one drop of juice pressed from the leaves contained 100 – 150 of the needle-like crystals. The investigators developed dermatitis similar to that of the workers within an hour of contact with aqueous suspensions of the isolated crystals. Previously, Sakai et al (1984) determined that raphide crystals longer than 180 mm in length caused irritation. Salinas et al. (2001) further confirmed that irritation occurred only at body locations where workers had direct skin contact with the plants. When the raphide suspension was passed through single and double layered cotton cloth, 75 and 92% of the crystals, respectively, were removed. The authors proposed that clothing could be an effective barrier to the calcium oxalate raphides.

Outside of agave plantations and tequila distilleries, agave-induced irritant dermatitis is relatively rare (Ricks et al., 1999). Twelve cases of irritant contact dermatitis provoked by the popular ornamental plant, *Agave americana*, have been reported (Hackman et al. 2006). Ricks et al. (1999) presented a case report of Agave-induced purpura on the anterior legs in an otherwise healthy patient. The condition developed as a result of landscaping work during which an *A. americana* plant was cut down with a chain saw. Histopathology examination of punch biopsy was consistent with hypersensitivity vasculitis.

In Mexico, leaves of *A. lechugia* are used to make fibers used in ‘‘estropajo,’’ or scouring pads for washing dishes. Salinas et al. (2001) reported that when estropajos were used while bathing there were complaints about skin irritation. They examined estropajos purchased in local markets in Guadalajara, Jal., Mexico, and found raphides in all of the products examined. Raphides were also found in the leaves of *A. lechugia.*

Roots and leaves of various species of agave contain steroidal saponins, which vary in their biological activities. Santos et al. (1997) investigated the hemolytic activity of saponins which had been extracted and isolated from *Agave sisalana* leaf juice. Crude extracts from the leaves of *Agave americana* contain two utero-active compounds with properties similar to the neurotransmitter acetylcholine or other choline derivatives (Basilio et al. 1989). Steroids derived from the sisal plants *Agave sisilana* and *Agave americana* have been used in the preparation of antifertility agents anordin and dinordin (Crabbe, 1979). Bioactive materials that have been extracted and isolated from Agave plants have been studied extensively, however, the piñas were not the source of the material in any of these studies, nor was the blue Agave used.

Yokosuka et al. (2009) evaluated several steroidal saponins for their cytotoxic activity against HL-60 human promyelocytic leukemia cells. The saponins were isolated from hot methanol extracts of the whole plants of Agave utahensis. Relative to the etoposide control, furostanol saponins were non cytotoxic (IC50 >20 μg/mL) and spirostanol saponins were non- to moderately cytotoxic ( IC50 values of 5.5 to >20 μg/mL).

Ohtsuki et al. (2004) evaluated a chlorogenin hexasaccharide isolated from the leaves of Agave fourcroydes for its cytotoxic and cell cycle inhibitory activities. The isolated saponin was cytotoxic against HeLa cells, and showed a cell cycle inhibitory effect at the G2/M stage at the concentrations of 7.5 and 10 μg/mL, respectively.

The hecogenin saponins, including agavacides A and B, obtained from leaves of *Agave americana* were evaluated and found to have some antifungal activity against agricultural pathogens such as *Piricularia oryzae* and the human pathogenic yeast *Candida* species (Yang et al. 2006). The antifungal activity of the hecogenin saponins was found to be largely dependent on the composition of sugar moiety, and no activity was detected when the sugar moiety is less than four monosaccharide units.

The Agave plant was evaluated for its antimicrobial activity by Verástegui et al. (1996). The roots of Agave *lecheguilla* Torr. (Agavaceae) were extracted with ethanol and dried. The material was found to have the activity against several pathogenic bacteria and fungi with minimal inhibitory concentrations ranging from 3.3 – 12 mg/mL.

The extracts of several species, including *Agave americana* L. and *A. intermixta* Trel have reported anti-inflammatory activities. Lyophylized aqueous extracts of the leaves of *Agave americana* L. containing hecogenin and ticogenin were reported to have anti-inflammatory activity in rats at doses that did not harm the gastric mucosa (Peana et al. 1997).

Extracts of the leaves of *A. intermixta* Trel. were evaluated by Garcia et al. (2000) for anti-inflammatory activity. The estimated $LD_{50}$ in male albino mice (intraperitoneal) for an extract

from the leaf of *A. intermixta* Trel. was 543 ± 132 mg of dry residue / kg bw, equivalent to 19.3 ± 4.7 g of plant/kg bw. In the carrageenan-induced edema-rat paw model, oral administration of *A. intermixta* (300 and 500 mg/kg) produced a marked anti-inflammatory effect (81.4± 4.1% inhibition; $P<0.001$) which was comparable or greater than that of the reference compound, dexamethasone. Topical application (2 and 5 mg/mouse ear) also produced a 50% reduction in tetradecanoylphorbol acetate-induced edema in mice (Garcia et al., 2000).

Edible flowers from Agave salmiana (Maguey), were analyzed for nutritional content, Trypsin inhibitors and hemaglutinnins, alkaloids, saponins and cyanogenic glucosides (Sotelo et al. 2007). The studied flowers showed a good macronutrient composition and a high quality of essential amino acids. Cyanogenic glycosides were not detected in any of the flowers. But saponins, as expected, were present. Trypsin inhibitors in Agave salmiana flowers were measured at 1.11±0.10 Trypsin unit inhibited/mg sample; very low when compared with the content in most legume seeds. Also very low was the concentration of hemaglutinnins and agglutination observed (Sotelo et al. 2007).

An extract from the roots of *Agave lecheguilla* (amole) was been evaluated in human volunteers, as a potential treatment for patients with vitreous hemorrhage (Segura et al., 1996). Previously, a single dose (po) up to 6 g did not cause adverse effects (Garcia et al. 2000). Twelve healthy male volunteers, aged 25 – 35 participated in a short term study of oral amole. Prior to the beginning of the study, subjects were examined for clinical history, electrocardiogram, chest X-ray, sperm count and stool guaiac test for occult bleeding. Subjects were hospitalized during the treatment periods for daily medical examinations. Ten subjects were given 500 mg capsules of amole, at 12 hour intervals for 10 days, and two received control capsules. Clinical symptoms and blood chemistries, including glucose, creatinine, urea, cholesterol, uric acid, total protein, albumin, ALP, total bilirubin, direct bilirubin, ALT, AST, LDH, hematocrit, and hemoglobin, as well as white blood count and mean corpuscular hemoglobin concentration were assessed prior to the initiation of the treatments, after 10 days of treatment and at 90 days. There were no adverse gastric, cardiovascular or respiratory symptoms. There were no changes in the muscular and nervous systems. Blood glucose levels were slightly decreased after 10 days of treatment compared to levels in the same volunteers 10 days following the end of the treatment period; however changes remained within the normal range, and were not significantly different from the placebo controls.

**Table A-1.** S**aponins identified in *Agave*** *

| Source | Compound | Reference |
|---|---|---|
| Agave americana leaves | Three known saponins and bisdesmosidic spirostanol saponin, (25R)-3 β, 6 α-dihydroxy-5 α-spirostan-12-one 3,6-di-O-β-D-glucopyranoside | Yokosuka, A.; Mimaki, Y.; Kuroda, M. et al. A new steroidal saponin from the leaves of Agave americana. Planta Med. 2000;66(4):393-396. |
| Agave americana methanolic extract of leaves | Two new saponins, agavasaponin E and agavasaponin H Agavasaponin E is 3-O-[[beta]-d-xylopyranosyl-(1-->2glc1)-[alpha]-l-rhamnopyranosyl-(1-->4)-[alpha]-l-rhamnopyranosyl-(1-->3glc 1)-[beta]-d-glucopyranosyl-(1-->4)-[beta]-d-glucopyranosyl-(1-->4)-[alpha]-d-galactopyranosyl]-(25R)-5[alpha]-spirostan-12-on-3[beta]-ol; agavasaponin H is 3-O-[[beta]-d-xylopyranosyl-(1-->2 glc 1)-[alpha]-l-rhamnopyranosyl-(1-->4)-[alpha]-l-rhamnopyranosyl-(1-->3 glc 1)-[beta]-d-glucopyranosyl-(1-->4)-[beta]-d-glucopyranosyl-(1-->4)-[beta]-d-galactopyranosyl]-26-O-[[beta]-d-glucopyranosyl]-(25R)-5[alpha]-furostan-12-on-3[beta],22[alpha],26-triol. | Wilkomirski, B., V.A. Bobeyko, P.K. Kintia 1975. New steroidal saponins of Agave americana, Phytochemistry 14 (12) 2657-2659. |

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| Agave attenuate leaves | Steroidal saponin: (3β,β,25S)-spirostan-3-ylO-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside | Mendes, T. P.; Silva, G. M.; da Silva, B. P. et al. 2004. A new steroidal saponin from Agave attenuata. Nat. Prod. Res. 18(2):183-188. |
| Agave attenuata Salm-Dyck leaves | Steroidal saponin: (3β, 5 β, 22alpha, 25S)-26-( β-D-glucopyranosyloxy)-22-methoxyfurostan -3-yl O- β-D-glucopyranosyl-(1-->2)- β-D-glucopyranosyl-(1-->2)-O-[ β-D-glucopyranosyl-(1-->3)]-β-D-glucopyranosyl-(1-->4)-β-D-galactopyranosi de | da Silva, B. P.; de Sousa, A. C.; Silva, G. M. et al. A new bioactive steroidal saponin from Agave attenuata. *Z. Naturforsch. C.* 2002;57(5-6):423-428. |
| Agave aurea, A. avellanidens, A. cerulata, A. cerulata ssp. subcerulata, A. cocui, A. goldmaniana, A. shawii leaves | hecogenin and tigogenin were the major sapogenins isolated. Gitogenin was found in the extracts of all the leaf samples, except that of A. shawii, and manogenin and 9-dehydromanogenin in all but that of A. cocui. Chlorogenin was isolated from A. cocui, but was not detected in any of the other species examined. Qualitative and quantitative variations were found in the sapogenin contents of extracts of different regions of the same leaves of A. cocui and F. macrophylla. In particular, hecogenin predominated in the basal regions and tigogenin in the apical. | Blunden, G., A. Carabot, C. K. Jewers 1980. Steroidal sapogenins from leaves of some species of Agave and Furcraea, Phytochemistry 19 (11) 2489-2490. |
| Agave cantala fruits | steroidal glycoside, agaveside D: 3 β-(α-L-rhamnopyranosyl-(1→2),β-D-glycopyranosyl- (1→3)-β-D-glucopyranosyl[β-D-xylopyransoyl-(1→4)-α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl)-25R-5 α-spirostane | Uniyal, G. C.; Agrawal, P. K.; Sati, O. P. et al. 1991. A spirostane hexaglycoside from Agave cantala fruits. Phytochemistry 30(12):4187-4189 |
| Agave cantala fruits | agaveside A and B 3 beta-O-[beta-D-xylopyranosyl-(1----2),beta-D-xylopyranosyl-(1----3), beta-D-glucopyranosyl-(1----3)-[beta-D-xylopyranosyl-(1----3)-beta-D-galactopyranosyl-(1----2)]-beta-D-glucopyranosyl]-(25R)-5 alpha- spirostane and 3 beta-O-[beta-D-xylopyranosyl-(1----2), beta-D-xylopyranosyl-(1----3)-beta-D-glucopyranosyl-(1----3)- [beta-D-galactopyranosyl-(1----2)]-beta-D-glucopyranosyl]-(25R)-5 alpha- spirostane. | Uniyal GC Agrawal PK Thakur RS Sati OP 1990. Steroidal glycosides from Agave cantala. In: Phytochemistry 29(3):937-40 |
| Agave cantala fruits | agaveside C: 3[beta]-{[alpha]-l-rhamnopyranosyl-(1-->2)-[beta]-d-glucopyranosyl-(1-->3)-[beta]-d-glucopyranosyl-[[beta]-d-xylopyranosyl-(1-->4)-[alpha]-l-rhamnopyranosyl-(1-->2)]-[beta]-d-glucopyranosyl}-2[alpha]-hydroxy-25R-5[alpha]-spirostane | Girish C. Uniyal, Pawan K. Agrawal, Raghunath S. Thakur, Om P. Sati, Agaveside C, a steroidal glycoside from Agave cantala, Phytochemistry, Volume 30, Issue 4, 1991, Pages 1336-1339, |
| Agave cantala aerial part | gitogenin-3-O-[beta]-D-glucopyranosyl (1 --> 3)-[beta]-D-glucopyranoside. | Jain, D.C. 1987. Gitogenin-3-O-[beta]-D-laminaribioside from the aerial part of Agave cantala, Phytochemistry 26(6) 1789-1790. |
| Agave cantala ethanolic extract of the roots | 3-O-[[beta]-d-glucopyranosyl]-6O-[[beta]-d-glucopyranosyl]-(25R)-5[alpha]-22[alpha]-O- spirostan-3[beta], 6[alpha]-diol. | Sharma, S.C. O.P. Sati, 1982. A spirostanol glycoside from Agave cantala, Phytochemistry 21(7) 1820-1821 |
| Agave decipiens methanolic extract of the leaves | 3-O-[alpha]-l-rhamnopyranosyl-(1-->2)-[[alpha]-l-rhamnopyranosyl-(1-->4)]-[beta]-d-glucopyranosyl-26-O-[beta]-d-glucopyranosyl-22[alpha]-methoxy-(25R)-furost-5-ene-3[beta],26-diol (1), neoruscogenin 1-O-[beta]-d-glucopyranosyl-(1-->3)-[[alpha]-l-rhamnopyranosyl-(1-->2)]-[beta]-d-glucopyranosyl-(1-->4)-[beta]-d-galactopyranoside (2), 1-O-[alpha]-l-rhamnopyranosyl-(1-->2)-[[alpha]-l-rhamnopyranosyl-(1-->4)]-[beta]-d-glucopyranosyl-26-O-[beta]-d-glucopyranosyl-22-O-methylfurosta-5,25(27)-diene-1[beta],3[beta],22,26-tetraol (3) and neohecogenin 3-O-[beta]-d-glucopyranosyl-(1-->3)-[[beta]-d-xylopyranosyl-(1-->3)-[beta]-d-xylopyranosyl-(1-->2)]-[beta]-d-glucopyranosyl-(1-->4)-[beta]-d-galactopyranoside (4). | Abdel-Gawad, M.M. El-Sayed, E.S. Abdel-Hameed 1999. Molluscicidal steroidal saponins and lipid content of Agave decipiens, Fitoterapia, 70 (4) 371-381. |
| Agave fourcroydes leaves | A new chlorogenin hexasaccharide saponin: chlorogenin 3-O-[[alpha]--rhamnopyranosyl-(1 --> 4)-[beta]--glucopyranosyl-(1 --> 3)-{[beta]--glucopyranosyl-(1 --> 3)-[beta]--glucopyranosyl-(1 --> 2)}-[beta]--glucopyranosyl-(1 --> 4)-[beta]--galactopyranoside] | Ohtsuki, T. T. Koyano, T.Kowithayakorn, S. Sakai, N.Kawahara, Y.Goda, N.Yamaguchi, M. Ishibashi 2004. New chlorogenin hexasaccharide isolated from Agave fourcroydes with cytotoxic and cell cycle inhibitory activities, Bioorganic & Medicinal Chemistry 12 (14) 3841-3845 |
| Agave lecheguilla Leaves, roots, and seeds | 1.0% smilagenin, dry matter basis. The roots contained about 1.0% total genin, of which about 80% was smilagenin and the rest gitogenin. The seeds contained 1.5 to 2% hecogenin with some manogenin | Wall ME Warnock BH Willaman JJ. 1962. Steroidal sapogenins. LXVIII. Their occurrence in Agave lecheguilla. Econ Bot 16:266-269 |

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| Agave lecheguilla Torrey leaves | two steroidal sapogenin diols: (25R)-spirost-5-ene-2 α, 3 β-diol (yuccagenin) and (25R)-5 β-spirostane-3 β , 6 α-diol (Ruizgenin) | Blunden, G.; Carabot, A.; Cripps, A. L. et al. 1980. Ruizgenin, a new steroidal sapogenin diol from Agave lecheguilla. Steroids 35(5):503-510 |
| Agave lechuguilla roots | $C_{27}H_{44}O_{12}$: Hydrolysis yields galactose and an end-sapogenin identical to that obtained from Yucca filamentosa | Johns C.O. et al. 1922. A saponin from Agave lechuguilla Torrey. Journal of Biological Chemistry 52:335-347. |
| Agave lophantha Schiede leaves | (25R)-5 β-spirostan-3 β-ol-3-O-(β-D-apiofuranosyl(1→4) β-D -glucopyranosyl(1→3)[ β-D-glucopyranosyl(1→2)] β-D -galactopyranoside) and 26-O- β-D-glucopyranosyl(25R)-5 β-furost-20(22)-ene-3 β, 26-diol-3-O-(β-D-xylopyranosyl(1→3)-[ β-D-glucopyranosyl(1→2)] β-D-galactopyranoside), | Abdel-Khalik, S. M.; Miyase, T.; Melek, F. R. et al. 2002. New steroidal saponins from Agave lophantha Schiede and their pharmacological evaluation. Pharmazie 57(8):562-566. |
| Agave shrevei Gentry leaves | Steroidal saponin: 26-(β-D-glucopyranosyloxy)-22-methoxy-3-(O-β-D-glucopyranosyl-(1-->2 )O-[O-β-D-glucopyranosyl-(1-->4)-O-[O-β-D-glucopyranosyl-(1-->6)]-O- β-D-glucopyranosyl(1-->4)-β-D-galactopyranosyl]oxy)-(3β, 5α, 25 R)-furostane | da Silva, B. P.; Parente, J. P. 2005. A new bioactive steroidal saponin from Agave shrevei. *Z. Naturforsch. C.* 60(1-2):57-62 |
| Agave shrevei leaves | Steroidal saponin: 3-[O- β-D-glucopyranosyl-(12)-O-[O-β-D-glucopyranosyl-(14)-O-[O-β-D-glucopyranosyl-(16)]-O-β-Dglucopyranosyl-(14)-β-D-galactopyranosyl)-oxy]-(3β, 5α,25R)-spirostane. | Pereira de Silva et al. 2006. A new steroidal saponin from Agave shrevei. Natural Products Research 20(4)385-390. |
| Agave sisalana leaves | Barbourgenin, a steroidal sapogenin | Blunden, G.; Patel, A. V.; Crabb, T. A. 1986. Barbourgenin, a new steroidal sapogenin from Agave sisalana leaves. *J. Nat. Prod.* 49(4):687-689 |
| *Agave sisalana* forma Dong No. 1 - dried fermented residues of leaf-juices | Five steroidal saponins, named dongnosides C (3), D (2), E (1) B (4) and A (5). | Ding, Y.; Tian R. H.; Yang, C. R. et al. 1993. Two new steroidal saponins from dried fermented residues of leaf-juices of Agave sisalana forma Dong No. 1. *Chem. Pharm. Bull. (Tokyo)* 41(3):557-560 |
| Agave sisalana form Dong No 1 methanol extracts of the fermented residues of leave-juices | Three new steroidal saponins, dongnosides E, D and C: tigogenin-3-O-[beta]-d-xylopyranosyl(1-->2)[[beta]-d-glucopyranosyl(1-->3)][beta]-d-glucopyranosyl(1-->4)[beta]-d-galactopyranoside, tigogenin-3-O-[beta]-d-xylopyranosyl(1-->3)[beta]-d-xylopyranosyl(1-->2)[[beta]-d-glucopyranosyl (1-->3)[beta]-d-glucopyranosyl(1-->4)[beta]-d-galactopyranoside and tigogenin-3-O-[alpha]-l-rhamnopyranosyl (1-->4)[beta]-d-xylopyranosyl(1-->2)[[beta]-d-glucopyranosyl (1-->3)][beta]-d-glucopyranosyl(1-->4)[beta]-d-galactopyranoside, respectively. | Ding Yi, Chen Yan-Yong, Wang De-Zu, Yang Chong-Ren, 1989. Steroidal saponins from a cultivated form of Agave sisalana, Phytochemistry,28 (10) 2787-2791 |
| Agave sislana | Saponin, Hecogenin (IV) was used as the starting material for cortisone manufacture. | Fazli, F. R. 1968. Contraceptives and other steroid drugs: their production from steroidal sapogenins. Pak. J. Sci. 20(1 and 2):64-67. |
| Agave sisalana Leaf extract and leaf juice | hecogenin 3[beta]-hydroxy-(25R)-5[alpha]-spirostan-12-one and tigogenin (25R)-5[alpha]-spirostan-3[beta]-ol | Cripps, A.L. and G. Blunden. 1978. A quantitative gas-liquid chromatographic method for the estimation of hecogenin and tigogenin in the leaves, juice and sapogenin concentrates of agave sisalana, Steroids 31(5) 661-669 |
| Agave utahensis whole plants | isolation of 15 steroidal saponins including five spirostanol saponins and three furostanol saponins | Yokosuka, A. and Mimaki, Y. 2009. Steroidal saponins from the whole plants of Agave utahensis and their cytotoxic activity. Phytochemistry,70(6)807-815. |

*Summarized and updated from Sigma Aldrich Life Science Nutrition Research learning center – plant profiler for *Agave sissalana:* http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler/agave.html accessed June 3, 2011 and updated from the current literature search.

### 9.3 Literature Search Strategy

The literature search strategy employed for this GRAS assessment on Inufib™ was based on the following search terms, as no Chemical Abstract Service Registry Number (CASRN) was available for agave inulin per se:

> Agave inulin, agave fructans, agavins, GRAS fructans, prebiotic fructans, fructans *Agave tequilana* Weber, agave fructosan, agave polyfructosan, agave

carbohydrates, *Agave tequilana* Weber var. *azul*, fructans and agave, GRAS fructans , fructans functional foods, prebiotic fructans agave.

As a minimum, the following data banks were searched:

- ChemID Plus
- Registry of Toxic Effects of Chemical Substances (RTECS)
- Hazardous Substances Data Bank (HSDB)
- GENE-TOX
- Environmental Mutagen Information Center (EMIC)
- Developmental and Reproductive Toxicology (DART)
- TOXLINE – Core and Special
- TRI (Toxics Release Inventory)
- Chemical Carcinogenesis Research Information System (CCRIS)
- Medline (via PubMed)
- Integrated Risk Information System (IRIS)
- Syracuse Research Corporation Online Toxic Substance Control Act Database (TSCATS)

The literature search for this chemical was initially conducted on April 13, 2011, updated on October 3, 2011, and updated again on April 8, 2015. This document includes all relevant information retrieved as a result of that search.

The FDA website with the search term "Agave" yielded 14 hits. All 14 entries were reviewed and the following items were considered relevant to this GRAS notification. Where appropriate they have been addressed within this document.

1. Five species of Agave are listed in the FDA poisonous plants database; *A. Americana*, *A. atrovirens* (maguey), *A. fourcroydes* (henequen), *A. sisalana* (sisal), and *Agave victoriae-reginae* [http://www.accessdata.fda.gov/scripts/Plantox/Detail.CFM?ID=5850] The poisonous constituents that have been characterized in Agave are primarily associated with the leaves and roots, as discussed within the document.

2. Agave nectar is an ingredient in The Xymogen Bars that were the subject of an FDA initiated recall because the Xymogen Bars may contain undeclared peanut protein (Enforcement report, August 24, 2011). The subject of this GRAS notice is agave inulin, not agave nectar.
   http://www.fda.gov/Safety/Recalls/EnforcementReports/ucm269605.htm

3. Two import alerts were reported on Oct 1, 2010 for agave inulin products described as "Fiber Agave Inulina" from the firm "Agaviotica S.A. De C.V.; Distrito B 4 No 433, Monterrey, Mexico." Products were subject to Detention without Physical Examination (DWPE) under this Import Alert (a.k.a. Red List) (unapproved new drug – misbranded drug). *41 B - - 99* Foods with Supplemental Nutrients Added, with or without Artificial Sweeteners; *62 B - - 99* Anti-Hyperlipidemic N.E.C. These alerts are related to label claims, and the firm charged is not the manufacturer of the product that is the subject of this GRAS notice.

4. There are import refusal reports for six agave products including 3 agave syrups, 2 agave honeys and 1 agave inulin. For all of the syrup and honey products, the violation was due to the presence of pesticides between August 2007 and January 2009 and the manufacturer listed was "Extrusiones Home S de RL De CV, Juan Valdivia 36, Col 5 De Mayo, Guadalajara, Mexico." The single charge made against the agave inulin (November 10, 2010) was "The article appears to be a new drug without an approved new drug application" and the manufacturer listed is Agaviotica S.A. De C.V.; Distrito B 4 No 433, Monterrey, Mexico." Products manufactured by "IIDEA" were not the subject of any of these violations.

5. Agave syrup from Mexico has been monitored for pesticide residues. No residues were found out of approximately 44 syrup samples monitored.

## 10.0 ATTACHMENTS

**10.1** Attachment 1

The iidea Company

Premium Agave Quality Products

Tlaquepaque, Jalisco, México. Sep 08, 2011

To whom it may concern,

This letter is to certify that our Inulin Premium (Inufib) has very low concentrations of saponins and terpenes according with an external analysis.

The External Laboratory concluded that the concentrations of this compounds are below of 0.1 ppm.

Sincerely,

The iidea Company
Premium Agave Quality Products
QUALITY ASSURANCE

[redacted]

Quality Manager

Periférico Sur No. 7750
Santa María Tequepexpan
C.P. 45601, Tlaquepaque, Jal., Mx.
Tel. 3003 4450

www.iidea.com.mx

**10.2** Attachment 2

VNIVERSIDAD NACIONAL
AVFNOMA DE
MEXICO

*FACULTAD DE QUÍMICA - UNAM*
*UNIDAD DE SERVICIOS DE APOYO A LA INVESTIGACIÓN*
*JEFATURA*

*U.S.A.I.*

*FQUI/USAI/468/2011*

*Asunto: informe.*

To whom it may concern:

This letter is to certify that one sample of Powder Inulin Premium (Inufib) identified with number lot 4NIPP11064 , was analyzed for the detection of saponins and terpenes using a method of extraction developed internally by our laboratory combined with a validated method for the analysis of organic compounds by GC/MS Clave: **PT-USAI-FQ-EM-001.**

Extraction procedure

To 5g of the sample 15 ml of hexane where added and submitted to sonication for 15 min. The suspension was filtered and the extract was evaporated under a nitrogen current until reducing the volume to 1mL.
From the extract 1 ml was injected to the GC/MS system.

GC/MS conditions

The sample was analyzed in GC/MS system from Agilent using a GC 5890 and a MS 5973 with a 5% phenyl-methyl silicon capillary column 30 m long, 250 µm of inner diameter and a film thickness of 0.25 µm. The oven started at 50°C kept at that temperature for 1 min and then it was programmed at 7°C/min rate up to 300°C and maintained for 5 min. The injection was performed under the split mode with a split ratio of 30:1. The carrier gas was Helium (99.999% PRAXAIR).
The mass spectrometer conditions where under Electron Impact Ionization at 70 eV and 300 µA using the scan mode and performing the scan function from 33 to 550 amu.

Although the analysis was not a quantitative determination (lack of standards) our laboratory routinely works with samples that require very low concentration determinations. Our system usually works on the ppb level (analysis of phthalates with a detection limit 7ppb). Because the sample was analyzed under these same conditions it is possible to assume that if the compounds ecogenin or ecognin were present in the sample it's concentration would be under 7ppb.

*Av. Universidad 3000, Cd. Universitaria, Circuito Escolar, Facultad de Química Edif. "B"*
*Tel.: Lab. 56.22.37.86, Ofna. 56.23.25.21 y Fax 56.23.25.22*
*e-mail: hgomez@servidor.unam.mx*

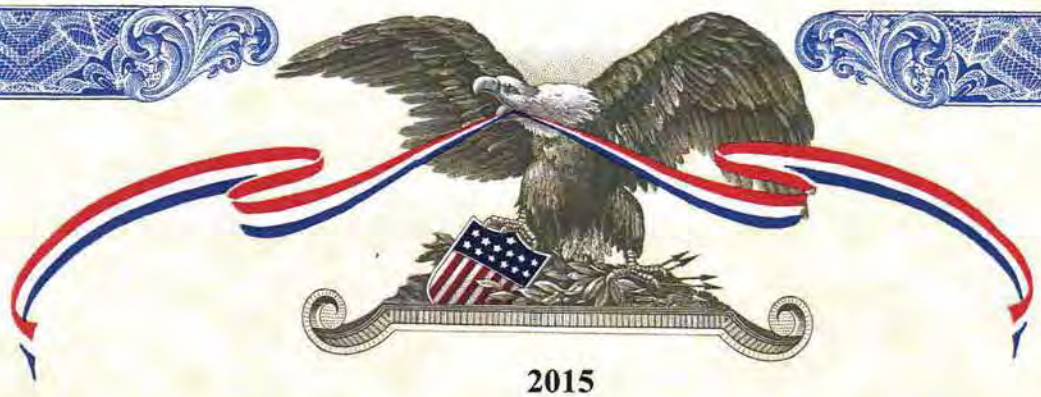
Our laboratory has performed in the past a lot of analysis of natural products (plant extracts) so we do have experience on the mass spectra interpretation of saponins. When we searched the chromatogram looking for mass spectra that might resemble the spectra of of ecogenin or ecognin we couldn't find one that even resembles these molecules.

ATENTAMENTE
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, México D. F., October 18, 2011.

(b) (6)

**M. C. HUMBERTO GOMEZ RUIZ**
**JEFE DE LA UNIDAD**

**10.3** Attachment 3



2015

# CERTIFICATE OF REGISTRATION

*This certifies that:*

**Industrializadora Integral Del Agave, SAPI de CV**
**Periferico Sur 7750, Colonia Santa Maria Tequepexpan**
**Tlaquepaque, JA 45601**
**Mexico**

is registered with the U.S. Food and Drug Administration pursuant to the Federal Food Drug and Cosmetic Act, as amended by the Bioterrorism Act of 2002 and the FDA Food Safety Modernization Act, such registration having been verified as currently effective on the date hereof by Registrar Corp:

| | |
|---|---|
| U.S. FDA Registration No.: | **13439186334** |
| U.S. Agent for FDA Communications: | **Registrar Corp**<br>144 Research Drive, Hampton, Virginia, 23666, USA<br>Telephone: +1-757-224-0177 • Fax: +1-757-224-0179 |

*This certificate affirms that the above stated facility is registered with the U.S. Food and Drug Administration pursuant to the Federal Food Drug and Cosmetic Act, as amended by the Bioterrorism Act of 2002 and the FDA Food Safety Modernization Act, such registration having been verified as effective by Registrar Corp as of the date hereof, and Registrar Corp will confirm that such registration remains effective upon request and presentation of this certificate until December 31, 2015, unless such registration has been terminated after issuance of this certificate. Registrar Corp makes no other representations or warranties, nor does this certificate make any representations or warranties to any person or entity other than the named certificate holder, for whose sole benefit it is issued. Registrar Corp assumes no liability to any person or entity in connection with the foregoing. The U.S. Food and Drug Administration does not issue a certificate of registration, nor does the U.S. Food and Drug Administration recognize a certificate of registration. Registrar Corp is not affiliated with the U.S. Food and Drug Administration.*

[redacted]

**Registrar Corp**

144 Research Drive, Hampton, Virginia, 23666, USA
Telephone: +1-757-224-0177 • Fax: +1-757-224-0179
info@registrarcorp.com • www.registrarcorp.com

**Russell K. Statman**
**Executive Director**
**Registrar Corp**
**Dated:** March 9, 2015

**10.4** Attachment 4

# HACCP PLAN – AGAVE INULIN

## 1.0 PURPOSE

To establish uniform guidelines to assure the administration, implementation and maintenance of Hazard Analysis Critical Control Point Program to reduce or prevent food safety hazards, so that only safe products of the highest quality are produced

## 2.0 SCOPE

The HACCP plan applies to processes and departments involved in the manufacturing of Agave inulin. All employees assigned to these areas shall follow the steps in-process to ensure safe product is manufactured and delivered to the customer.

## 3.0 RESPONSIBILITIES

It is the responsibility of the safety team leader and the operations director to enforce the monitoring process required for this HACCP plan, and that training has occurred for all responsible individuals assigned to Production.

It is the responsibility of the safety team leader to ensure training has been provided and to audit the HACCP plan.

## 4.0 OVERVIEW OF PREREQUISITE PROGRAMS

There are nine (9) key prerequisite programs that are essential for an adequate and effective HACCP plan. IIDEA has incorporated these key programs into the day-to-day operation of the facility.

### 4.1 Sanitation Program:

The sanitation program includes a Master Cleaning Schedule. This schedule encompasses the entire plant, including the exterior of the building. All items on the schedule are to be completed on a specified day, week and month, and verified by the HACCP Coordinator for completeness. All sanitation employees receive training before starting this function. The sanitation program can be found in the Production office

### 4.2 Good Manufacturing Practices (GMPs):

The GMP program includes daily GMPs to be followed. The audit used encompasses the requirements of AIB Consolidated Standards for Food Safety. All employees and contractors are trained on the requirements of GMPs, and documentation of such is maintained on file. The plant has a strict “no glass” policy. Hard plastic is also monitored. The GMP program is maintained by Quality Assurance.

## HACCP PLAN – AGAVE INULIN

### 4.3 Pest Control Program:
All pest control activities are performed by an outside contractor. Bait stations are used outside the building. Traps are used internally. The plant has a record of all contractor visits.

### 4.4 Chemical Control Program:
The chemical control program dictates that all chemicals are stored in secure locations separate from production areas. Access to these chemicals is limited to authorized personnel. MSDS books are located in several areas where the chemicals are commonly used.

### 4.5 Supplier control:
IIDEA follows a procedure for selection, evaluation and audit of authorized suppliers. All raw materials, packaging and finished goods transports are inspected. We also ratify our analytical methods with those of our suppliers.

### 4.6 Recall/Traceability Program:
All products manufactured and packaged by IIDEA are coded and identified for ease of recall in the event of a food safety issue. A mock recall procedure is performed at least once a year.

### 4.7 Quality System
IIDEA works according to a quality management system based in the SQF 2000 code. We have also deveoped, identified and follow our raw material specifications, packaging specifications and finished product specifications.

### 4.8 Production Teams
All products are manufactured according to standard opperating procedures, including methods for the verification and validation of our critical control points; metal detectors and instrument calibration.

### 4.9 Reception, Storage and Shipping
Finished products, raw materials, packaging materials and chemicals are stored according to good storage practices. Finished products are shipped according to good transport practices.

# HACCP PLAN – AGAVE INULIN

## 5.0 HACCP TEAM

| POSITION | NAME |
|---|---|
| Quality Manager<br>HACCP Coordinator | [redacted] |
| Operation Director | [redacted] |
| HACCP Assistant | [redacted] |
| Quality Control Chief | [redacted] |
| Human Resources | [redacted] |
| Maintenance | [redacted] |
| Supply chain Director | [redacted] |
| Purchasing | [redacted] |
| Traceability Chief | [redacted] |
| Biosecurity | [redacted] |

## 6.0 PRODUCT DESCRIPTION

6.1 Dry Inulin:

6.1.1 Physical Chemical Properties

| | |
|---|---|
| Humidity: | 0.5 – 4.0% |
| pH: | 4.0 – 6.0 |
| Density: | 0.6 – 0.8 g/ml |
| Color: | White, yellowish fine powder |
| Storage stability: | Stable, hygroscopic |
| Taste: | Slighlty sweet |

6.1.2 Product Specifications

| | |
|---|---|
| Ash content: | < 5.0 % |
| Dry matter: | ≥ 98.0 % carbohydrates |
| Composition: | ≥ 88.0 % Inulin |
| | ≤ 10.0 % Fructose |
| | ≤ 3.5 % Glucose |
| | ≤ 2.0 % disaccharides |
| | Mesofilic: ≤ 2,500 UFC/g |
| Microbiological | Coliform: ≤ 10 UFC/g |
| Contaminats: | Yeast and molds: ≤ 100 UFC/g |

6.1.3 Intended Use:

As a bulking agent or ingredient in a great variety of foods and the cosmetic industry.

**HACCP PLAN – AGAVE INULIN**

6.1.4 Packing
25 kg bag
30 kg bag

6.1.5 Shelf Life
3 years from manufacturing date

6.2 Liquid Inulin

6.2.1 Physical Chemical Properties

| | |
|---|---|
| Humidity: | 27 – 31% |
| Concentration: | 69º – 73º Brix |
| pH: | 3.5 – 6.0 |
| Density: | 1.34 – 1.36 g/ml |
| Color: | 300 – 1000 icumsa |
| Storage stability: | Stable, hygroscopic |
| Taste: | Slighlty sweet |

6.2.2 Product Specifications:

| | |
|---|---|
| Ash content: | < 0.7 % |
| Dry matter: | ≥ 98.0 % carbohydrates |
| Composition: | ≥ 80.0 % Inulin |
| | ≤ 15.0 % Fructose |
| | ≤ 5.0 % Glucose |
| | ≤ 2.0 % disaccharides |
| | Mesofilic: ≤ 2,500 UFC/g |
| Microbiological | Coliform: ≤ 10 UFC/g |
| Contaminats: | Yeast and molds: ≤ 100 UFC/g |

6.2.3 Intended Use
As a bulking agent or ingredient in a great variety of foods and the cosmetic industry

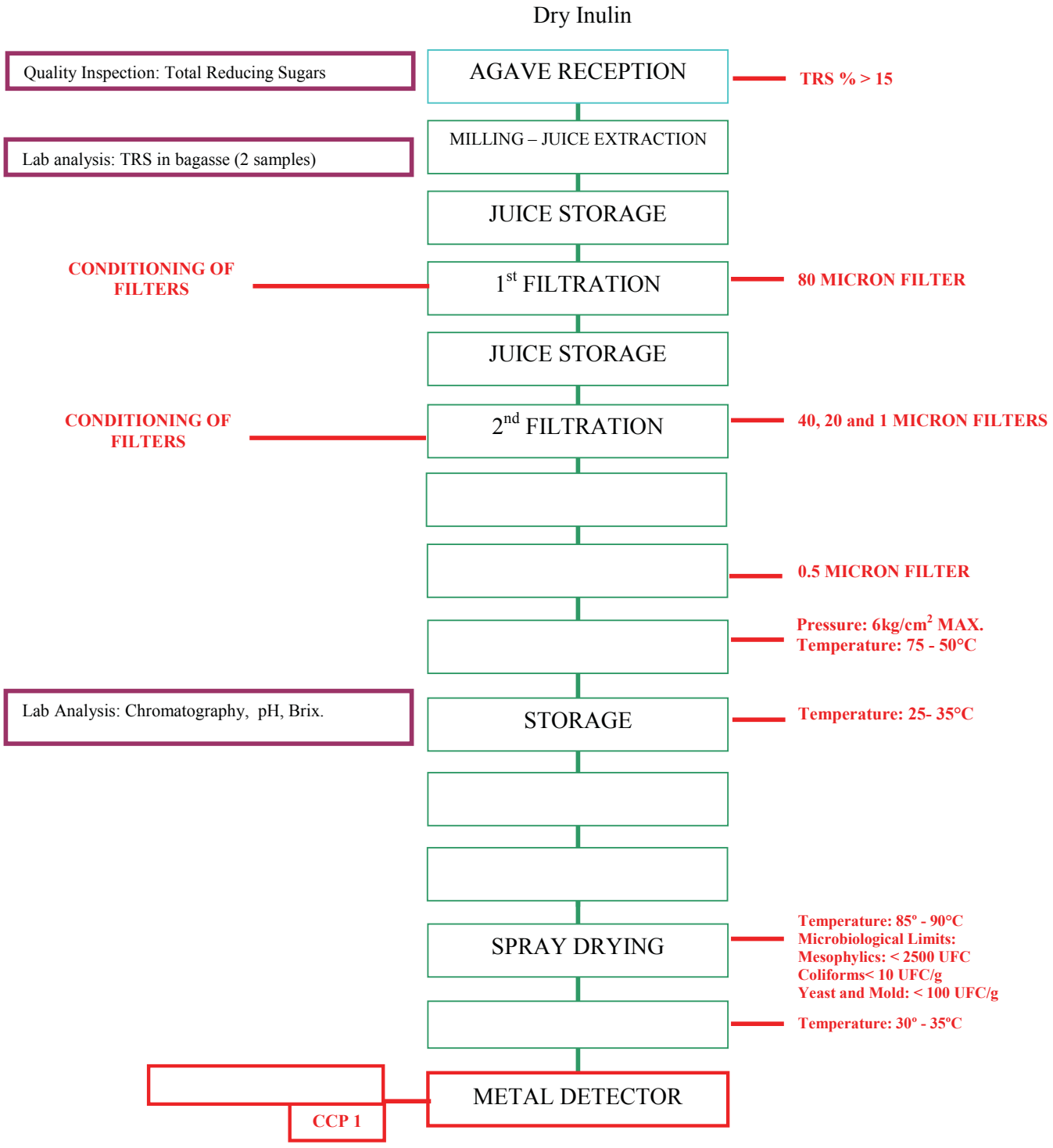
6.2.4 Packing
IBC, drums.

6.2.5 Shelf life
3 months from manufacturing date.

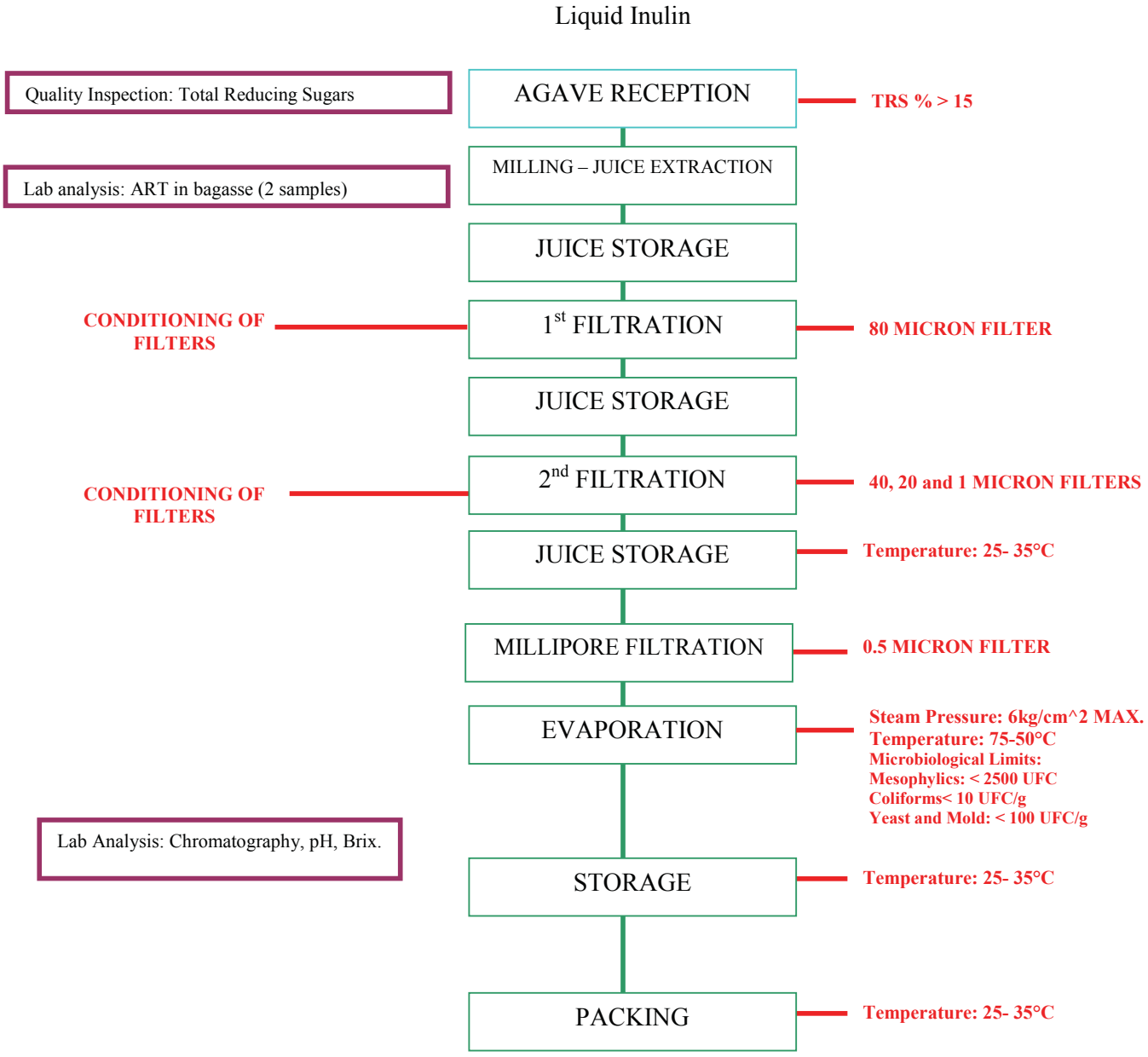
Industrializadora Integral del Agave SA de CV

## HACCP PLAN – AGAVE INULIN

### 7.0 FLOW DIAGRAM



# HACCP PLAN – AGAVE INULIN



## HACCP PLAN – AGAVE INULIN

## 8.0 PROCESS DESCRIPTION

7.1 Dry Inulin

| Step | Process | Description |
| --- | --- | --- |
| 1 | Agave Reception | Quality control department checks the appereance of the product and determine the total reducing sugars percentage (TRS %) taking a representative sample of 5 out of every 200 agave "piñas" from each lot. The agave is then released for its unloading if the TRS% value is above 15%. The lot is rejected otherwise. |
| 2 | Milling – Juice Extraction | Agave "piñas" are led to a conveyour that transports them into a mill and a series of extractors. The product is sieved and squeezed. The juice falls into tubs while the resulting bagasse is separated and transported into a container. |
| 3 | Juice Storage | Extracted juice is pumped from the tubs through a series of pipelines and into storage tanks of "raw" juice. |
| 4 | 1st filtration | Juice is filtrated by means of a press filter to eliminate suspended solids (media size: 80 micron) |
| 5 | Juice Storage | Once filtered, the juice is conducted to storage tanks until enough product is stored to continue to the next process |
| 6 | 2nd filtration | The juice passes through a series of press filters using a filter aid (perlite) to eliminate suspended solids. Media sizes: 40, 20 and 1 micron. |
| 7 | Juice Storage | Once filtered the juice is conducted to storage tanks until enough product is stored to continue to the next process |
| 8 | Millipore filtration | The juice passes through a third filter to eliminate suspended solids and microorganisms. Media size: 0.5 micron. |
| 9 | Evaporation | The juice is concentrated using a triple effect evaporator until it reaches a concentration between 60º and 65º Brix. Using a steam pressure of 6 $kg/cm^2$ and a temperature between 75 and 50ºC |
| 10 | Storage | Once concentrated, the product is conducted to storage tanks until enough is stored to continue to the next process. |
| 11 | Packing (liquid) | The product is bottled in containers |
| 12 | Storage | The product is drained in tanks until enough is |

| | | |
| --- | --- | --- |
| | | stored to continue to the next process. |
| 13 | Spray Drying | The product passes through a spray drier, that operates at a maximum temperature between 85º and 90ºC |
| 14 | Packing | The dried product is conducted through a series of pipelines into a hopper and then it is bagged and sealed. |
| 15 | Metal detector | The product is validated for metal residues. |

7.2 Liquid Inulin

| Step | Process | Description |
| --- | --- | --- |
| 1 | Agave Reception | Quality control department checks the appereance of the product and determine the total reducing sugars percentage (TRS %) taking a representative sample of 5 out of every 200 agave "piñas" from each lot. The agave is then released for its unloading if the TRS% value is above 15%. The lot is rejected otherwise. |
| 2 | Milling – Juice Extraction | Agave "piñas" are led to a conveyour that transports them into a mill and a series of extractors. The product is sieved and squeezed. The juice falls into tubs while the resulting bagasse is separated and transported into a container. |
| 3 | Juice Storage | Extracted juice is pumped from the tubs through a series of pipelines and into storage tanks of "raw" juice. |
| 4 | 1st filtration | Juice is filtrated by means of a press filter to eliminate suspended solids (media size: 80 micron) |
| 5 | Juice Storage | Once filtered, the juice is conducted to storage tanks until enough product is stored to continue to the next process |
| 6 | 2nd filtration | The juice passes through a series of press filters using a filter aid (perlite) to eliminate suspended solids. Media sizes: 40, 20 and 1 micron. |
| 7 | Juice Storage | Once filtered the juice is conducted to storage tanks until enough product is stored to continue to the next process |
| 8 | Millipore filtration | The juice passes through a third filter to eliminate suspended solids and microorganisms. Media size: 0.5 micron. |
| 9 | Evaporation | The juice is concentrated using a triple effect evaporator until it reaches a concentration between |

| | | |
|---|---|---|
| | | 65º and 70º Brix. Using a steam pressure of 6 kg/cm2 and a temperature between 75º and 50ºC |
| 10 | Storage | Once filtered, the product is conducted to storage tanks until enough is stored to continue to the next process. |
| 11 | Packing | The product is bottled in different size containers according to our clients needs. |

## 8.0 INGREDIENT HAZARD ANALYSIS

| List all ingredients used in the product process, or plant | Identify known hazards | Likely Risk (likehood & severity) H = high, M = medium, L = low | | Basis for the decision | Identify Prerequisite Programs or process steps to reduce or eliminate known hazards |
|---|---|---|---|---|---|
| | | Likehood | Severity | | |
| Bag | B – N/A | | | | |
| | C – N/A | | | | |
| | P – Foreign Matter | L | L | Storage, transport and manufacturing conditions | Transport verification procedure, Supplier approval and evaluation |
| IBC and others | B – N/A | | | | |
| | C – N/A | | | | |
| | P – Foreign Matter | L | L | Storage, transport and manufacturing conditions | Transport verification procedure, Supplier approval and evaluation |
| Filter Aid | B – N/A | | | | |
| | C – N/A | | | | |
| | P – Foreign Matter | L | L | Storage, transport and manufacturing conditions | Certificate of analysis of the product and supplier guarantee |
| Agave | B - Salmonella and coliforms | L | H | Raw materials comes from organic fields | Supplier control, good agricultural practices |
| | C – Pesticides | L | H | Raw materials comes from organic fields | Supplier control, good agricultural practices |
| | P - Foreign Matter | H | L | Process conditions | Visual inspection in the milling area, filtration process |

## HACCP PLAN – AGAVE INULIN

| (1) List each process step from the Process Flow Diagram. (For Receiving only, bring forward each Ingredient Hazard Analysis that was determined to be a critical Ingredient.) | (2) Does this ingredient or process step INTRODUCE a potential food safety hazard. Identify here. (Be as specific as possible when listing the hazard) | (3) Is this hazard CONTROLLED by a Prerequisite Program or process step? If YES, identify the Program or process. If a Prerequisite program or process is identified, do not complete Columns 4-6 and go to next process step. If NO, go to Column 4 | (4) Is this hazard ELIMINITAED by a subsequent (later) process step? If YES, this step is NOT a CCP. Identify the subsequent process step in Column 5 and proceed to the next process step. If the hazard is eliminated at this step (no subsequent elimination step) enter NO and go to Column 6 and assign a CCP number. | (5) Identify the last process step that will eliminate the potential hazard (Example: metal detector, filter, etc.) | (6) Assign a CCP number when the answer in Column 4 is NO. Otherwise leave blank. |
|---|---|---|---|---|---|
| Agave reception | B - Salmonella and coliforms | Millipore Filtration, Supplier control, good agricultural practices | | | |
| | C - Pesticides | Organic Certificate of fields | | | |
| | P - Foreign Matter | 1st Filtration | | | |
| Milling - Juice Extraction | B - Salmonella and coliforms | Millipore Filtration, GMP's | | | |
| | C - Lubricant oil | Maintenance, GMP's | | | |
| | P - Foreign Matter | 1st Filtration | | | |
| Juice Storage | B – Salmonella and coliforms | Millipore Filtration, GMP's | | | |
| | C - Sanitation Products | GMP's, Sanitation program, Chemical control program | | | |
| | P - Foreign Matter | 1st Filtration | | | |
| 1st Filtration | B – Salmonella and coliforms | Millipore Filtration, GMP's | | | |
| | C - Sanitation Products | GMP's, Sanitation program, Chemical control program | | | |
| | P - Foreign Matter | 2nd Filtration | | | |
| Juice Storage | B – Salmonella and coliforms | Millipore Filtration, GMP's | | | |
| | C - Sanitation Products | GMP's, Sanitation program, Chemical control program | | | |
| | P - Foreign Matter | 2nd Filtration | | | |

## HACCP PLAN – AGAVE INULIN

| | | | | | |
|---|---|---|---|---|---|
| 2nd Filtration | B – Salmonella and coliforms | Millipore Filtration, GMP's | | | |
| | C - Sanitation Products | GMP's, Sanitation program, Chemical control program | | | |
| | P - Foreign Matter | Millipore Filtration | | | |
| Juice Storage | B – Salmonella and coliforms | Millipore Filtration, GMP's | | | |
| | C - Sanitation Products | GMP's, Sanitation program, Chemical control program | | | |
| | P - Foreign Matter | Millipore Filtration | | | |
| Millipore Filtration | B - Salmonella and coliforms | Evaporation, GMP's | | | |
| | C - Sanitation Products | GMP's, Sanitation program, Chemical control program | | | |
| | P – Metal Residues | Metal Detector | | | |
| Evaporation | B - Salmonella and coliforms | Spray Drying, GMP's | | | |
| | C - Sanitation Products | GMP's, Sanitation program, Chemical control program | | | |
| | P – Metal Residues | Metal Detector | | | |
| Storage | B - Salmonella and coliforms | Spray Drying, GMP's | | | |
| | C - Sanitation Products | GMP's, Sanitation program, Chemical control program | | | |
| | P – Metal Residues | Metal Detector | | | |
| Packing (liquid) | B - Salmonella and coliforms | Spray Drying, GMP's | | | |
| | C - Sanitation Products | GMP's, Sanitation program, Chemical control program | | | |
| | P – Metal Residues | Metal Detector | | | |

## HACCP PLAN – AGAVE INULIN

| | | | | | |
|---|---|---|---|---|---|
| Storage | B - Salmonella and coliforms | Spray Drying, GMP's | | | |
| | C - Sanitation Products | GMP's, Sanitation program, Chemical control program | | | |
| | P – Metal Residues | Metal Detector | | | |
| Spray Drying | B – N/A | | | | |
| | C - Sanitation Products | GMP's, Sanitation program, Chemical control program | | | |
| | P – Metal Residues | Metal Detector | | | |
| Packing (dry) | B – N/A | | | | |
| | C - Sanitation Products | GMP's, Sanitation program, Chemical control program | | | |
| | P – Metal Residues | Metal Detector | | | |
| Metal Detector | B – N/A | | | | |
| | C – N/A | | | | |
| | P - Metal residues | No | No | | **CCP 1** |

## HACCP PLAN – AGAVE INULIN

### 9.0 HACCP MASTER PLAN

| # PCC | PCC | Hazard | Critical limits | Monitoring | | Corrective Action | Verification | Records |
|---|---|---|---|---|---|---|---|---|
| 1 | Metal Detector | Metal residues | 7 mm | *What* | Metal residues | When a product with metal is detected the line must be stopped and it should be tested again. If the detection is confirmed, the product will be segregated and disposed of according to the non-conformity procedure. | Quality Analyst must verified and register the correct operation of metal detector every startup and end of the production. | Metal detector control (FREN05) Certificate of validation of metal detector (annual) |
| | | | | *How* | Validate with contaminants 0.8 mm Fe, 0.8 mm N Fe, 1.2 mm SS | | | |
| | | | | *When* | Every two hours | | | |
| | | | | *Who* | Operator | | | |

## HACCP PLAN – AGAVE INULIN

### 10.0 DEFINITIONS

**Hazard Analysis Critical Control Point (HACCP) –** Assessment of the process to identify any reasonable potential hazards associated with the process, or rework.

**Critical Control Point (CCP)** – Any step where significant hazards can be controlled to prevent, eliminate, or be reduced to acceptable levels.

**Biological Hazard** – Source of hazard in relationship to bacterial pathogens that may result in personal injury.

**Chemical Hazard** – Uncontrolled use or application of chemicals that may result in personal injury.

**Physical Hazard** – Any potentially harmful extraneous matter not normally found in finished product.

### 11.0 CHANGE CONTROL

| Version | Approval date | Modification | Approbed by |
|---|---|---|---|
| 01 | August 31, 2010 | Initial Version | Food Safety leader |
| 02 | March 10, 2011 | Revision and actualization | HACCP Coordinator |

### 12.0 APPROVALS

| [redacted] | [redacted] |
|---|---|
| Quality Control Chief | Quality Manager |
| Review | Approve |

### 13.0 DISTRIBUTION LIST

| #CC | Responsible | Signature | Date |
|---|---|---|---|
| 08 | Quality Control Chief | [redacted] | 10-03-11 |

## **10.5** Attachment 5

Global Standards
certification

# Certificate of Compliance

## Industrialidadora Integral del Agave, S.A. de C.V.

Periférico Sur 7750, Sta. María Tequepexpan
C.P. 45601 Tlaquepaque, Jalisco. México.

Has successfully implemented and passed the Certification Assesment and found its Hazard Analysis and Critical Control Points System in compliance with the requirements detailed bellow:

## HACCP / Good Manufacturing Practices (GMP)

**Scope: Manufacturing, Storage, Packaging and Sale of Agave Syrup.**

| | |
|---|---|
| **Certificate Number:** | **GSCHACCPMX-109** |
| **Initial Registration Date:** | **June 23, 2010** |
| **Registration Date:** | **June 23, 2010** |
| **Registration Period:** | **June 23, 2010 to June 22, 2013** |
| **Certification Scheme:** | **Single Site** |

(b) (6)

Executive Director

Global Standards
HACCP COMPLIANCE CERTIFICATE

Global Standards, S.C. Pedro Moreno 1677 Piso 4 -3 Col. Americana C.P. 44160 Guadalajara, Jalisco. México.

*adding-value to your business*

Global Standards
COMPANY CERTIFICATE

000101

**10.6** Attachment 6

SILLIKER®
Food Safety & Quality Solutions

# *Audit Recognition*

## Industrializadora Integral del Agave SA de CV (IIDEA):
## Tlaquepaque, Jalisco, Mexico

Completed a
Silliker Good Manufacturing Practices and Food Safety Systems Audit
With a score of

# 94.2%

| 2/21/2011 | (b) (6) | (b) (6) |
|---|---|---|
| Audit Date | Division Vice President | Chief Scientific Officer |

With over 40 years of experience and part of the Mérieux Alliance group of companies,
Silliker provides services that help assure food safety and nutrition worldwide.

**10.7** Attachment 7

# Bolsas Filtrantes Selladas *Accufit* de Nylon

Las bolsas filtrantes *Accufit Welded* de nylon de Filtration Systems son bolsas de superficie grado absoluto, específicamente fabricadas para altos contenidos de sólidos. Son una excelente opción para clarificar agua, químicos, pinturas, resinas, recubrimientos y pegamentos. Son filtros que no liberan ningún tipo de fibra y que además pueden soportar altas temperaturas.


La media filtrante que atrapa los contaminantes en la superficie del filtro o bien en alguna parte de la tortuosa matriz de fibras por la que atraviesa el fluido, está diseñada con una alta precisión en el micraje especificado y está integrada por varias capas ultrasónicamente selladas y laminadas con la tecnología patentada de Filtration Systems, todas ellas integradas a un cuello de zero bypass.
Estas bolsas son muy útiles para altos flujos y ofrecen una pérdida de presión sumamente baja. Estos filtros de superficie son lo opuesto de un filtro de profundidad ya que la mayor parte de los contaminantes son retenidos en la superficie de las capas filtrantes. Esta característica les permite atrapar grandes concentraciones de sólidos sin perder su permeabilidad, es decir, el flujo no disminuye en gran medida aunque se hayan atrapado grandes concentraciones de contaminantes.

Una de las grandes características que hacen único a este filtro, es que puede ser modificado para satisfacer una amplia variedad de condiciones de filtración de líquidos; Por ejemplo, un gran rendimiento combinado con una retención de sólidos en micrajes pequeños.

## Datos Técnicos

| | |
|---|---|
| Media Filtrante | Nylon N6 |
| Cuello | Nylon Zero-Bypass |
| Acabado | Ultrasónicamente Selladas y Laminadas |
| No. de Capas | Múltiples. De 2 a 12 |
| Cambio Recomendado | 30 psid |
| Temperatura Máxima | 180 °C |
| Caída Máxima de Presión | 30 psi |
| Dirección del Flujo | De Adentro hacia Afuera |
| Micrajes | Absolutos |
| FDA | Aprobadas para Alimentos y Bebidas |
| Presentación | Empacadas Individualmente |
| Medidas | 7”x16”, 7”x32”, 4”x14” y 4”x24” |

**Diseño**

**Tecnología**

**Rendimiento**

## Aplicaciones

- Pinturas
- Adhesivos
- Resinas
- Emulsiones
- Recubrimientos
- Ceras
- Derivados de Petróleo
- Tintas
- Aceites
- Solventes
- Agua
- Químicos

Filtration Systems

serco COMERCIAL S.A. DE C.V.

Guillermo Prieto 943 Ote. - Monterrey, N.L. c.p. 64000 Tel. (81)-83-45-24-59 Fax (81)-83-44-08-87
Aguascalientes • Guadalajara • Hermosillo • México • Torreón • Monterrey • Querétaro
www.serco.com.mx
serco@serco.com.mx

SCB-27-001

**10.8** Attachment 8

# GRUPO CENCON CENTRO DE CONTROL S.A. DE C.V.

ALIMENTOS • BEBIDAS • MEDICAMENTOS • AGUAS • COSMÉTICOS • AGROINDUSTRIA
DESARROLLO DE PRODUCTOS • INDUSTRIA QUÍMICA EN GENERAL

FORMAT MPA-F-032A-01-0

## TEST REPORT

**ANÁLISIS**

**Microbiología:**
Cuenta Bacteriana
Grupo Coliforme
Salmonella
Estafilococos
Estreptococos
E. Coli
Hongos
Levaduras
V. Cholerae
Anaerobios
Otros

**Fisicoquímicos:**
Bromatólogicos
Minerales
Vitaminas
Aditivos
Aflatoxinas
Materia Extraña
Otros

**Instrumentales:**
Cromatografía de Gases
Cromatografía de Líquidos
Absorción Atómica
Espectrofotometría
Infrarrojo
Aminogramas
Otros

**Aguas:**
Bacteriológicos
Fisicoquímicos
Aguas Residuales

**Asesorías:**
Control de Calidad
Inspecciones Sanitarias
Auditorias de Calidad
Desarrollo de Productos
Investigación Aplicada
Estudios Especiales
Restaurantes y Comedores
FDA Registrar
Unidades de Verificación

**Otros Análisis:**
Biodegradabilidad
Biodisponibilidad

(b) (4)
January 21st, 2011
O. T. 1827
Sample 1/6
Page 1/1

**INDUSTRIALIZADORA INTEGRAL**
**DEL AGAVE, S.A. DE C.V.**
PERIFÉRICO SUR No. 7750
SANTA MARÍA TEQUEPEXPAN
TLAQUEPAQUE, JAL, C.P. 45601

**"INULINA EN POLVO LOTE: 4NIPP103365"**

| ASSAY | ANALYTICAL RESULTS |
|---|---|
| Account of aerobes mesophyll | 150* UFC/g |
| Total coliforms | Less than 10* UFC/g |
| Fungi | Less than 10* UFC/g |
| Yeast | Less than 10* UFC/g |
| *Salmonella sp* (in 25g) | Absence (Negative) |

* Estimated Value

| ASSAY | METHOD REFERENCE |
|---|---|
| Account of aerobes mesophyll | NOM-092-SSA1-1994 this essay we realize in agar for standard account incubated for 48 h to 35ºC. |
| Total coliforms | NOM-113-SSA1-1994 this essay we realize in agar bile and violet red incubated to 35ºC for 24 ± 2 h. |
| Fungi<br>Yeast | NOM-111-SSA1-1994 this essay we realize in agar potato dextrose acidified incubated for 25 ºC ± 1 ºC for 5 days. |
| *Salmonella sp* | NOM-114-SSA1-1994 |

GRUPO CENCON
(b) (6)
I.A. NALLELY HERNANDEZ ALVAREZ
GENERAL MANAGER
Q.F.B. LUCILA TRIGUEROS DÍAZ
CHEMICAL ANALYST OF MICROBIOLOGY

*jger

LOS RESULTADOS REPORTADOS AMPARAN ÚNICAMENTE LA MUESTRA ANALIZADA
Y NO NECESARIAMENTE EL LOTE QUE REPRESENTA

Av. Galileo Galilei 4299 Fracc. Arboledas C.P. 45070 Guadalajara, Jalisco, México **www.cencon.com.mx**
Tels: (0133) 3634-5640 / 3634-7210 / 3632-1912 Fax: (0133) 3632-1461 **grupocencon@prodigy.net.mx** R.F.C. GCC081201GZ1

# GRUPO CENCON CENTRO DE CONTROL S.A. DE C.V.

ALIMENTOS • BEBIDAS • MEDICAMENTOS • AGUAS • COSMÉTICOS • AGROINDUSTRIA
DESARROLLO DE PRODUCTOS • INDUSTRIA QUÍMICA EN GENERAL

FORMAT MPA-F-032A-01-0

## TEST REPORT

(b) (4) [redacted]

January 21th, 2011
O. T. 1827
Sample 2/6
Page 1/1

**ANÁLISIS**

**Microbiología:**
Cuenta Bacteriana
Grupo Coliforme
Salmonella
Estafilococos
Estreptococos
E. Coli
Hongos
Levaduras
V. Cholerae
Anaerobios
Otros

**Fisicoquímicos:**
Bromatólogicos
Minerales
Vitaminas
Aditivos
Aflatoxinas
Materia Extraña
Otros

**Instrumentales:**
Cromatografía de Gases
Cromatografía de Líquidos
Absorción Atómica
Espectrofotometría
Infrarrojo
Aminogramas
Otros

**Aguas:**
Bacteriológicos
Fisicoquímicos
Aguas Residuales

**Asesorías:**
Control de Calidad
Inspecciones Sanitarias
Auditorias de Calidad
Desarrollo de Productos
Investigación Aplicada
Estudios Especiales
Restaurantes y Comedores
FDA Registrar
Unidades de Verificación

**Otros Análisis:**
Biodegradabilidad
Biodisponibilidad

**INDUSTRIALIZADORA INTEGRAL DEL AGAVE, S.A. DE C.V.**
PERIFÉRICO SUR No. 7750
SANTA MARÍA TEQUEPEXPAN
TLAQUEPAQUE, JAL, C.P. 45601

**"INULINA EN POLVO LOTE: 4NIPP11004"**

| ASSAY | ANALYTICAL RESULTS |
|---|---|
| Account of aerobes mesophyll | 90* UFC/g |
| Total coliforms | Less than 10* UFC/g |
| Fungi | Less than 10* UFC/g |
| Yeast | Less than 10* UFC/g |
| *Salmonella sp* (in 25g) | Absence (Negative) |

* Estimated Value

| ASSAY | METHOD REFERENCE |
|---|---|
| Account of aerobes mesophyll | NOM-092-SSA1-1994 this essay we realize in agar for standard account incubated for 48 h to 35ºC. |
| Total coliforms | NOM-113-SSA1-1994 this essay we realize in agar bile and violet red incubated to 35ºC for 24 ± 2 h. |
| Fungi | NOM-111-SSA1-1994 this essay we realize in agar potato dextrose acidified incubated for 25 ºC ± 1 ºC for 5 days. |
| Yeast | |

GRUPO CENCON
(b) (6) [redacted]
I.A. NALLELY HERNANDEZ ALVAREZ
GENERAL MANAGER
Q.F.B. LUCILA TRIGUEROS DÍAZ
CHEMICAL ANALYST OF MICROBIOLOGY

*jger

LOS RESULTADOS REPORTADOS AMPARAN ÚNICAMENTE LA MUESTRA ANALIZADA
Y NO NECESARIAMENTE EL LOTE QUE REPRESENTA

Av. Galileo Galilei 4299 Fracc. Arboledas C.P. 45070 Guadalajara, Jalisco, México **www.cencon.com.mx**
Tels: (0133) 3634-5640 / 3634-7210 / 3632-1912 Fax: (0133) 3632-1461 **grupocencon@prodigy.net.mx** R.F.C. GCC081201GZ1

The i Company
Premium Agave Quality Products

***PRODUCT:*** ***ORGANIC AGAVE POWDER INULIN 100% BLUE WEBBER***

| | |
|---|---|
| ***AMOUNT*** | ***Samples*** |
| ***LOT:*** | (b) (4) |
| ***PACK DATA*** | ***20-ene-11*** |
| ***USE BEFORE*** | ***20-ene-14*** |

## *CERTIFICATE CHEMICAL ANALYSIS*

| ***ASSAY*** | | ***SPECIFICATIONS*** | ***METHOD*** |
|---|---|---|---|
| ***APPEARENCE*** | CREAMY WHITE FINE POWDER | | *ORGANOLEPTIC ANALYSIS* |
| ***FRUCTOSE*** | *4.62* | ***3,0 - 10,0%*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***GLUCOSE*** | *0.71* | ***Max. 3,5 %*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***SACCHAROSE*** | *1.18* | ***Max. 2,0%*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***INULIN*** | *91.47* | ***88,0 - 94,0 %*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***OTHER CARBOHYDRATES*** | *1.15* | ***Max. 6,0 %*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***TOTAL CARBOHYDRATES*** | *99.13* | ***Min. 98,0 %*** | *METHOD HPLC HP 1100 - HP 1200* |

***ANALYZE***

[redacted]

The i Company
Premium Agave Quality Products

**INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. De C.V**

**QUALITY ASSURANCE**

**INTERNAL REPORT OF MICROBIOLOGICAL VERIFICATION**

Date 20/01/2011
FOLIO 003/11

# Lot (b) (4)

Amount Samples

| Analysis | Results | Specifications | Units | Method | |
|---|---|---|---|---|---|
| Total Count | 13 | Max. 2,500 | CFU/g | AOAC 2002.07 | NOM-092-SSA1 * |
| Coliform | < 10 | Max. 10 | CFU/g | AOAC 2005.03 | NOM-113-SSA1* |
| Yeast | < 10 | Max. 100 | CFU/g | AOAC 2002.11 | NOM-111-SSA1* |
| Mold | < 10 | Max. 100 | CFU/g | AOAC 2002.11 | NOM-111-SSA1* |
| Salmonella | Absent | Absent | in 25 gr. | - | NOM-114-SSA1* |

* Mexican regulations

[redacted]

The i Company
Premium Agave Quality Products

***PRODUCT:*** ***ORGANIC AGAVE POWDER INULIN 100% BLUE WEBBER***

| | |
|---|---|
| ***AMOUNT*** | ***Samples*** |
| ***LOT:*** | (b) (4) |
| ***PACK DATA*** | ***28-sep-10*** |
| ***USE BEFORE*** | ***28-sep-13*** |

***CERTIFICATE CHEMICAL ANALYSIS***

| ***ASSAY*** | | ***SPECIFICATIONS*** | ***METHOD*** |
|---|---|---|---|
| ***APPEARENCE*** | CREAMY WHITE FINE POWDER | | *ORGANOLEPTIC ANALYSIS* |
| ***FRUCTOSE*** | *5.66* | ***3,0 - 10,0%*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***GLUCOSE*** | *0.60* | ***Max. 3,5 %*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***SACCHAROSE*** | *0.69* | ***Max. 2,0%*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***INULIN*** | *90.98* | ***88,0 - 94,0 %*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***OTHER CARBOHYDRATES*** | *1.09* | ***Max. 6,0 %*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***TOTAL CARBOHYDRATES*** | *99.02* | ***Min. 98,0 %*** | *METHOD HPLC HP 1100 - HP 1200* |

***ANALYZE***

[redacted]

The i Company
Premium Agave Quality Products

**INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. De C.V**

**ASEGURAMIENTO DE LA CALIDAD**

**INTERNAL REPORT OF MICROBIOLOGICAL VERIFICATION**

Date 28/09/2010
FOLIO 006/10

# Lot (b) (4)

Amount Samples

| Analysis | Results | Specifications | Units | Method | |
|---|---|---|---|---|---|
| Total Count | 260 | Max. 2,500 | UFC/g | AOAC 2002.07 | NOM-092-SSA1 * |
| Coliform | < 10 | Max. 10 | UFC/g | AOAC 2005.03 | NOM-113-SSA1* |
| Yeast | < 10 | Max. 100 | UFC/g | AOAC 2002.11 | NOM-111-SSA1* |
| Mold | < 10 | Max. 100 | UFC/g | AOAC 2002.11 | NOM-111-SSA1* |
| Salmonella | Absent | Absent | in 25 gr. | - | NOM-114-SSA1* |

* Mexican regulations

[redacted]

Analyze

The i Company
Premium Agave Quality Products

***PRODUCT:*** ***ORGANIC AGAVE POWDER INULIN 100% BLUE WEBBER***

| | |
|---|---|
| ***AMOUNT*** | ***Samples*** |
| ***LOT:*** | (b) (4) |
| ***PACK DATA*** | ***10-feb-10*** |
| ***USE BEFORE*** | ***10-feb-13*** |

## *CERTIFICATE CHEMICAL ANALYSIS*

| ***ASSAY*** | | ***ESPECIFICATIONS*** | ***METHOD*** |
|---|---|---|---|
| ***APPEARENCE*** | CREAMY WHITE FINE POWDER | | *ORGANOLEPTIC ANALYSIS* |
| ***FRUCTOSE*** | *4.73* | ***3,0 - 10,0%*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***GLUCOSE*** | *2.32* | ***Max. 3,5 %*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***SACCHAROSE*** | *0.93* | ***Max. 2,0%*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***INULIN*** | *90.00* | ***88,0 - 94,0 %*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***OTHER CARBOHYDRATES*** | *1.91* | ***Max. 6,0 %*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***TOTAL CARBOHYDRATES*** | *99.89* | ***Min. 98,0 %*** | *METHOD HPLC HP 1100 - HP 1200* |

***ANALYZE***

[redacted]

The i Company
Premium Agave Quality Products

**INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. De C.V**

**QUALITY ASSURANCE**

**INTERNAL REPORT OF MICROBIOLOGICAL VERIFICATION**

Date 10/02/2010
FOLIO 003/10

# Lot (b) (4)

Amount Samples

| Analysis | Results | Specifications | Units | Method | |
|---|---|---|---|---|---|
| Total Count | 10 | Max. 2,500 | UFC/g | AOAC 2002.07 | NOM-092-SSA1 * |
| Coliform | < 10 | Max. 10 | UFC/g | AOAC 2005.03 | NOM-113-SSA1* |
| Yeast | < 10 | Max. 100 | UFC/g | AOAC 2002.11 | NOM-111-SSA1* |
| Mold | < 10 | Max. 100 | UFC/g | AOAC 2002.11 | NOM-111-SSA1* |
| Salmonella | Absent | Absent | in 25 gr. | - | NOM-114-SSA1* |

* Mexican regulations

[redacted]

Analyze

The i Company
Premium Agave Quality Products

***PRODUCT:*** ***ORGANIC AGAVE POWDER INULIN 100% BLUE WEBBER*** ***P***

| | |
|---|---|
| ***AMOUNT*** | ***Samples*** |
| ***LOT:*** | (b) (4) |
| ***PACK DATA*** | ***23-ene-11*** |
| ***USE BEFORE*** | ***23-ene-14*** |

## *CERTIFICATE CHEMICAL ANALYSIS*

| ***ASSAY*** | | ***SPECIFICATIONS*** | ***METHOD*** |
|---|---|---|---|
| ***APPEARENCE*** | CREAMY WHITE FINE POWDER | | *ORGANOLEPTIC ANALYSIS* |
| ***FRUCTOSE*** | *4.96* | ***3,0 - 10,0%*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***GLUCOSE*** | *0.45* | ***Max. 3,5 %*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***SACCHAROSE*** | *0.60* | ***Max. 2,0%*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***INULIN*** | *91.98* | ***88,0 - 94,0 %*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***OTHER CARBOHYDRATES*** | *1.03* | ***Max. 6,0 %*** | *METHOD HPLC HP 1100 - HP 1200* |
| ***TOTAL CARBOHYDRATES*** | *99.02* | ***Min. 98,0 %*** | *METHOD HPLC HP 1100 - HP 1200* |

***ANALYZE***

[redacted]

The i Company
Premium Agave Quality Products

**INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. De C.V**

**QUALITY ASSURANCE**

**INTERNAL REPORT OF MICROBIOLOGICAL VERIFICATION**

Date 23/01/2011
FOLIO 005/11

# Lot (b) (4)

Amount Samples

| Analysis | Results | Specifications | Units | Method | |
|---|---|---|---|---|---|
| Total Count | 10 | Max. 2,500 | CFU/g | AOAC 2002.07 | NOM-092-SSA1 * |
| Coliform | < 10 | Max. 10 | CFU/g | AOAC 2005.03 | NOM-113-SSA1* |
| Yeast | < 10 | Max. 100 | CFU/g | AOAC 2002.11 | NOM-111-SSA1* |
| Mold | < 10 | Max. 100 | CFU/g | AOAC 2002.11 | NOM-111-SSA1* |
| Salmonella | Absent | Absent | in 25 gr. | - | NOM-114-SSA1* |

* Mexican regulations

[redacted]
Analyze

**10.9** Attachment 9

The i Company
Premium Agave Quality Products

***PRODUCT:*** ***ORGANIC AGAVE LIQUID INULIN 100% BLUE WEBBER***

| | |
|---|---|
| ***AMOUNT*** | ***Samples*** |
| ***LOT:*** | (b) (4) |
| ***PACK DATA*** | ***11-ene-11*** |
| ***USE BEFORE*** | ***11-abr-11*** |

## *CERTIFICATE CHEMICAL ANALYSIS*

| ***ASSAY*** | | ***METHOD*** |
|---|---|---|
| ***APPEARENCE*** | LIGHT AMBER | *ORGANOLEPTIC ANALYSIS* |
| ***pH*** | *4.10* | *NMX-FF-110-SCFI* |
| ***BRIX*** | *60.4* | *NMX-FF-110-SCFI* |
| ***FRUCTOSE*** | *4.54* | *METHOD HPLC HP 1100 - HP 1200* |
| ***GLUCOSE*** | *1.65* | *METHOD HPLC HP 1100 - HP 1200* |
| ***SACCHAROSE*** | *1.11* | *METHOD HPLC HP 1100 - HP 1200* |
| ***INULIN*** | *90.00* | *METHOD HPLC HP 1100 - HP 1200* |
| ***OTHER CARBOHYDRATES*** | *1.62* | *METHOD HPLC HP 1100 - HP 1200* |
| ***CARBOHYDRATES TOTAL*** | *98.92* | *METHOD HPLC HP 1100 - HP 1200* |

***ANALYZE***

[redacted]

The i Company
Premium Agave Quality Products

**INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. De C.V**

**ASEGURAMIENTO DE LA CALIDAD**

**INTERNAL REPORT OF MICROBIOLOGICAL VERIFICATION**

Date 11/01/2011
FOLIO 001/11

# Lot (b) (4)

Amount Samples

| Analysis | Results | Units | Method | |
|---|---|---|---|---|
| Total Count | 359 | CFU/g | AOAC 2002.07 | NOM-092-SSA1 * |
| Coliform | < 10 | CFU/g | AOAC 2005.03 | NOM-113-SSA1* |
| Yeast | < 10 | CFU/g | AOAC 2002.11 | NOM-111-SSA1* |
| Mold | < 10 | CFU/g | AOAC 2002.11 | NOM-111-SSA1* |

[redacted]

Analyze

The i Company
Premium Agave Quality Products

***PRODUCT:*** ***ORGANIC AGAVE LIQUID INULIN 100% BLUE WEBBER***

| | |
|---|---|
| ***AMOUNT*** | ***Samples*** |
| ***LOT:*** | (b) (4) |
| ***PACK DATA*** | ***14-ene-11*** |
| ***USE BEFORE*** | ***14-abr-11*** |

## *CERTIFICATE CHEMICAL ANALYSIS*

| ***ASSAY*** | | ***METHOD*** |
|---|---|---|
| ***APPEARENCE*** | LIGHT AMBER | *ORGANOLEPTIC ANALYSIS* |
| ***pH*** | *4.00* | *NMX-FF-110-SCFI* |
| ***BRIX*** | *63.2* | *NMX-FF-110-SCFI* |
| ***FRUCTOSE*** | *3.13* | *METHOD HPLC HP 1100 - HP 1200* |
| ***GLUCOSE*** | *1.57* | *METHOD HPLC HP 1100 - HP 1200* |
| ***SACCHAROSE*** | *1.09* | *METHOD HPLC HP 1100 - HP 1200* |
| ***INULIN*** | *89.89* | *METHOD HPLC HP 1100 - HP 1200* |
| ***OTHER CARBOHYDRATES*** | *2.59* | *METHOD HPLC HP 1100 - HP 1200* |
| ***CARBOHYDRATES TOTAL*** | *98.27* | *METHOD HPLC HP 1100 - HP 1200* |

***ANALYZE***

[redacted]

The i Company
Premium Agave Quality Products

**INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. De C.V**

**ASEGURAMIENTO DE LA CALIDAD**

**INTERNAL REPORT OF MICROBIOLOGICAL VERIFICATION**

Date 14/01/2011
FOLIO 002/11

# Lot (b) (4)

Amount Samples

| Analysis | Results | Units | Method | |
|---|---|---|---|---|
| Total Count | 338 | CFU/g | AOAC 2002.07 | NOM-092-SSA1 * |
| Coliform | < 10 | CFU/g | AOAC 2005.03 | NOM-113-SSA1* |
| Yeast | < 10 | CFU/g | AOAC 2002.11 | NOM-111-SSA1* |
| Mold | < 10 | CFU/g | AOAC 2002.11 | NOM-111-SSA1* |

[redacted]
Analyze

The i Company
Premium Agave Quality Products

***PRODUCT:*** ***ORGANIC AGAVE LIQUID INULIN 100% BLUE WEBBER***

| | |
|---|---|
| ***AMOUNT*** | ***Samples*** |
| ***LOT:*** | (b) (4) |
| ***PACK DATA*** | ***15-ene-11*** |
| ***USE BEFORE*** | ***15-abr-11*** |

## *CERTIFICATE CHEMICAL ANALYSIS*

| ***ASSAY*** | | ***METHOD*** |
|---|---|---|
| ***APPEARENCE*** | LIGHT AMBER | *ORGANOLEPTIC ANALYSIS* |
| ***pH*** | *4.05* | *NMX-FF-110-SCFI* |
| ***BRIX*** | *60.4* | *NMX-FF-110-SCFI* |
| ***FRUCTOSE*** | *4.59* | *METHOD HPLC HP 1100 - HP 1200* |
| ***GLUCOSE*** | *0.50* | *METHOD HPLC HP 1100 - HP 1200* |
| ***SACCHAROSE*** | *0.62* | *METHOD HPLC HP 1100 - HP 1200* |
| ***INULIN*** | *89.63* | *METHOD HPLC HP 1100 - HP 1200* |
| ***OTHER CARBOHYDRATES*** | *2.70* | *METHOD HPLC HP 1100 - HP 1200* |
| ***CARBOHYDRATES TOTAL*** | *98.04* | *METHOD HPLC HP 1100 - HP 1200* |

***ANALYZE***

[redacted]

The i Company
Premium Agave Quality Products

**INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. De C.V**

**ASEGURAMIENTO DE LA CALIDAD**

**INTERNAL REPORT OF MICROBIOLOGICAL VERIFICATION**

Date 15/01/2011
FOLIO 003/11

# Lot (b) (4)

Amount Samples

| Analysis | Results | Units | Method | |
|---|---|---|---|---|
| Total Count | 414 | CFU/g | AOAC 2002.07 | NOM-092-SSA1 * |
| Coliform | < 10 | CFU/g | AOAC 2005.03 | NOM-113-SSA1* |
| Yeast | < 10 | CFU/g | AOAC 2002.11 | NOM-111-SSA1* |
| Mold | < 10 | CFU/g | AOAC 2002.11 | NOM-111-SSA1* |

[redacted]
Analyze

**10.10** Attachment 10

| Analyses | Laboratory | Turnaround time |
|---|---|---|
| **Nutritional Labelling**<br>Total fat<br>Total Dietary Fiber<br>Energy values according to EC 90/496<br>Energy values according to EC 2008/100<br>Moisture<br>Ash<br>Proteins<br>Carbohydrates content<br>Fatty extraction<br>Fatty acid composition<br>Fatty acids in 100 g product calculation<br>Sodium<br>Extraction for HPLC / IC sugar analyses<br>Sugar profile | Eurofins Analytics France | 5 weeks |
| **Fructans** | | |
| **PCB, Dioxine** | | |
| **Heavy metals**<br>Arsenic<br>Lead<br>Mercury<br>Cadmium | Silliker | 20 working days |
| **Pesticides** | | |
| **Shelf life studies** | Silliker | 30 days - 2 months |

**10.11** Attachment 11

Industrializadora Integral del Agave SA de CV

# TECHNICAL DATA SHEET

## PRODUCT: POWDER INULIN PREMIUM

| SECTION 1: PRODUCER DATA | |
|---|---|
| Name of the company: | Industrializadora Integral del Agave SA de CV |
| Address: | Av. Periférico Sur 7750, Tlaquepaque Jalisco, México. |
| Telephone: | +52 (33) 3003-4450 |

| SECTION 2: MICROBIOLOGIC CHARACTERISTICS AND PHYSICAL-CHEMICAL DATA | |
|---|---|
| Appearance | White powder |
| Aroma | Neutral |
| Moisture | 0.5 – 4.0 % |
| pH (1%) | 4.0 – 6.0 |
| Ash | Máx. 5.0 % |
| Inulin: | 88.0 – 94.0% |
| Fructose: | 3.0 – 10.0 % |
| Glucose: | Máx. 3.5 % |
| Sucrose: | Máx. 2.0% |
| Carbohydrates Total: | 98.0 – 100.0 % |
| Other carbohydrates: | Máx. 6.0% |
| Mesofilics: | Máx. 2,500 UFC |
| Coliforms: | Máx. 10 UFC |
| Yeast and Mold: | Máx. 100 UFC |

| SECTION 3: HAZARDOUS INGREDIENTS | |
|---|---|
| List: | 1. The product does not contain any hazardous ingredient or substance. |

| SECTION 4: RISK OF FIRE OR EXPLOSION | |
|---|---|
| Method of Fire extinction: | Chemical dust, $CO_2$ preferably |
| Cautions: | Do not expose the product to temperatures higher than 300°F |
| Ignition Temperature: | 300°F |

| SECTION 5: REACTIVITY | |
|---|---|
| Stability: | Stable |
| Incompatible Materials: | Strong Oxidants, flames. |
| Dangerous decompositions per component: | N/A |
| Conditions to avoid: | Do not overheat; reduce heat if the product begins to produce smoke. |

Industrializadora Integral del Agave SA de CV

# TECHNICAL DATA SHEET

## PRODUCT: POWDER INULIN PREMIUM

| SECTION 6: HEALTH RISK | |
|---|---|
| | |
| Routes: inhalation - N/A cutaneous – N/A ingestion – No hazardous | |
| Cancerigenous characteristics: | No |
| Exposure Health Damage Symptoms: | None |
| Exposure General medical conditions: | None |
| Applicable First Aid Procedures: | N/A |

| SECTION 7: PERSONAL PROTECTION | |
|---|---|
| Respiratory Protection: | Not necessary under normal use and handling. If dispersion of dust in the air using mouth covers. |
| Ocular Protection: | Use protection glasses on spilling cases to avoid splashing. If there is ocular contact, wash abundantly with clean water. |
| Hygiene Requirements: | Handle the product under the Good Manufacturing Practices and /or specific food regulations. |

| SECTION 8: USE AND HANDLING CAUTIONS | |
|---|---|
| In case of Spilling: | Do not step on the product, you may slip. Wash the area with water, once clean dry the surface. |
| Disposal handling: | Consult local regulations on disposal handling of food products. |
| Storage and Handling: | Handle the product with caution, avoid spilling. Store the product in cool places at room temperature; avoid overheating, highly hygroscopic product. |
| Shelf Life | 3 years |

**10.12** Attachment 12

(b) (4)

# Shelf life Study

# "AGAVE INULIN POWDER"

## Prepared for:

## INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. de C.V.

**Periférico Sur 7750. Santa Maria Tequepexpan, Tlaquepaque Jalisco**
**C.P: 45601 Tel.: 33 30 03 45 56**

[redacted]

## May de 2011

**Silliker México, S.A. de C.V.**

Carlos B. Zetina 138 Col Tacubaya
C.P. 11870 Deleg. Miguel Hidalgo, México, D.F.
Tel. +52 (55) 52.73.50.77 Fax: 26.14.11.42
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Tels: 01 (44) 22.16.16.33, 22.16.16.23
e-mail: liliana.lechuga@silliker.com.mx

## 1.- OBJETIVE

- ✓ Estimate agave inulin powder shelf life under ambient temperature (19-25 º C) and stressed temperatures of 35 º C and 45 º C, using sensory attributes loss as detrimental indicators,
- ✓ Determine the behavior of moisture as physical-chemical indicators.
- ✓ Initial microbiological analysis for Salmonella and E. coli analysis are also done
- ✓ Initial and final analysis for total coliformes, total plate count, yeasts and molds are also included.

## 2.- MATERIAL AND METHODS

Samples were identified individually in their commercial presentation (kraft packaging). They were placed in temperature controlled chambers: ambient temperature (19-25 º C, in a cool, dry and free from sunlight), 35 º C and 45 º C. Temperatures were recorded with a calibrated C- hygrometer / thermometer (Mod 10-95 Digital 355119-020).

- ❖ The moisture analysis was performed by Mexican Official Standard N0M-116-SSA1-1994.
- ❖ The total plate count was performed by Mexican Official Standard NOM-092-SSA1-1994
- ❖ Total Coliformes analysis was performed by Mexican Official Standard NOM-113-SSA1-1994
- ❖ The analysis of molds and yeasts was performed by Mexican Official Standard NOM-111-SSA1-1994
- ❖ The analysis of E. coli was performed using the method in CCAYAC-004-M-2006.
- ❖ Salmonella analysis was performed by Mexican Official Standard NOM-114-SSA1-1994

Sensory performance was conducted with seven trained panelists (trained to perceive different deterioration degrees when compared to an original sample), testing was for a 32 days period, using a structured 10 points scale "SENSORY SCALE LEVELS":

Level 10.0-8.0: Characteristic. The product has the taste, smell and original appearance as the initial sample or reference
Level 7.9–6.0: Acceptable. The product has undergone just perceptible changes in taste, smell and appearance, without being disagreeable.
Level 5.9-4.0: Marginal. The product has undergone slightly changes in taste or odor (slightly rancid or bitter), and / or color significantly different from the original.
Level 3.9-0.0: Unacceptable. The product has undergone noticeable changes in taste or odor (rancid or bitter), and color is significantly different from the original.

Sensory attributes identified as part of the customer's needs were valuated as:

- Appearance
- Color
- Odor
- Flavor
- Fluidity
- Rancidity

# 3 RESULTS

## 3.1 Sensory analysis

Sensory average scores are shown in the following table, taking into consideration time (days) and sensory attributes losses

***Table 1. Panelists average results at different temperatures: ambient (19 - 25 ° C), 35 º C and 45 º C***

| | AMBIENT | | | | | | 35ºC | | | | | | 45ºC | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Days | Appearance | Color | Odor | Flavor | Fluidity | Rancidity | Appearance | Color | Odor | Flavor | Fluidity | Rancidity | Appearance | Color | Odor | Flavor | Fluidity | Rancidity |
| 22-Mar | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| 1-Apr | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 9.8 | 10.0 | 9.9 | 10.0 | 9.8 | 9.9 | 9.6 | 9.7 | 9.8 | 10.0 |
| 8-Apr | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 9.9 | 9.7 | 9.7 | 9.9 | 10.0 | 10.0 | 9.7 | 9.4 | 9.6 | 9.7 | 9.3 | 9.9 |
| 15-Apr | 9.8 | 9.8 | 9.8 | 9.8 | 9.8 | 9.8 | 9.8 | 9.8 | 9.8 | 9.8 | 9.7 | 9.7 | 9.4 | 9.4 | 9.6 | 9.3 | 9.4 | 9.4 |
| 26-Apr | 9.7 | 9.7 | 9.6 | 9.6 | 9.6 | 9.7 | 9.7 | 9.2 | 9.5 | 9.3 | 9.5 | 9.5 | 9.3 | 8.4 | 9.0 | 8.7 | 9.0 | 9.1 |

***Note, Day one corresponds to a "fresh" reference, therefore the highest score ("10") is given. The "fresh" product represent o the best alternative presented by the client***

Sensory loss (sensory level log) in relation to time (days) is done trough a first-order kinetics, which consists of evaluating the detrimental loss (deterioration as a Log Y) versus time:

$$Log_{10}\, Y = (m * t) + I \qquad \text{equation (1)}$$

Where:

Y: is sensory loss (according to the hedonic scale sensory levels) based on a Log 10 scale.
m: is the slope
t: is the time
I: is the intercept

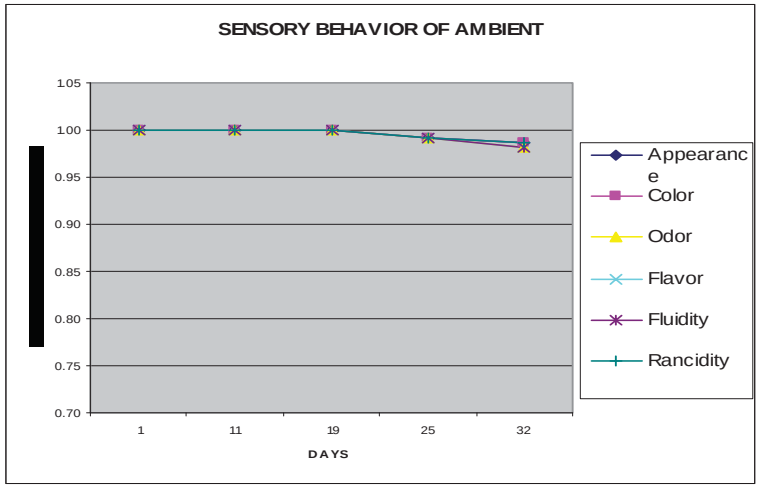
***From the log Y values table 2 is obtained.***

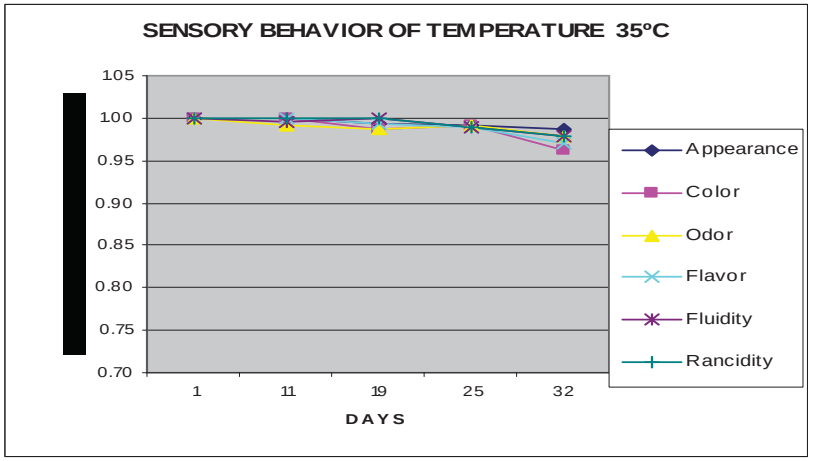
***Table2. Sensory analysis log at different temperatures: ambient, 35, 45 º C***
***Slope values (m), intercept (I)***

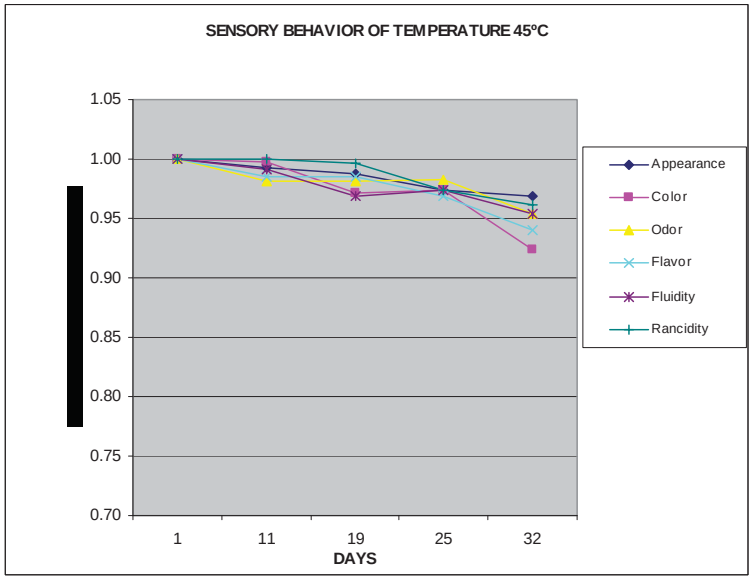
| | AMBIENTE | | | | | | 35ºC | | | | | | 45ºC | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Days | Appearance | Color | Odor | Flavor | Fluidity | Rancidity | Appearance | Color | Odor | Flavor | Fluidity | Rancidity | Appearance | Color | Odor | Flavor | Fluidity | Rancidity |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 11 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.00 | 0.99 | 1.00 | 0.99 | 1.00 | 0.98 | 0.99 | 0.99 | 1.00 |
| 19 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 0.99 | 0.99 | 0.99 | 1.00 | 1.00 | 0.99 | 0.97 | 0.98 | 0.99 | 0.97 | 1.00 |
| 25 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.97 | 0.97 | 0.98 | 0.97 | 0.97 | 0.97 |
| 32 | 0.99 | 0.99 | 0.98 | 0.98 | 0.98 | 0.99 | 0.99 | 0.96 | 0.98 | 0.97 | 0.98 | 0.98 | 0.97 | 0.92 | 0.95 | 0.94 | 0.95 | 0.96 |
| m | -0.0004 | -0.0004 | -0.0006 | -0.0006 | -0.0006 | -0.0004 | -0.0004 | -0.0011 | -0.0006 | -0.0009 | -0.0006 | -0.0007 | -0.0011 | -0.0022 | -0.0012 | -0.0017 | -0.0014 | -0.0013 |
| Int | 1.0032 | 1.0032 | 1.0047 | 1.0047 | 1.0047 | 1.0032 | 1.0022 | 1.0071 | 0.9999 | 1.0063 | 1.0033 | 1.0055 | 1.0030 | 1.0127 | 1.0006 | 1.0066 | 1.0030 | 1.0092 |
| R2 | 0.7209 | 0.7209 | 0.6925 | 0.6925 | 0.6925 | 0.7209 | 0.9134 | 0.6989 | 0.7892 | 0.7607 | 0.6685 | 0.7051 | 0.9463 | 0.7988 | 0.7655 | 0.8516 | 0.9202 | 0.7790 |

Table 2 presents the decline of sensory attributes at different temperatures (Figures 1, 2 and 3).

***Figures1, 2 and 3 integrates the sensory loss, time (days) and temperature (ambient (19-25 ° C), 35 and 45 º C***



Hedonic level 7 was established as the acceptable sensory loss limit; Level 7.9–6.0 is within the acceptance range. The product has undergone just perceptible changes in taste, smell and appearance, without being disagreeable.

From Equation 1, Shelf life time (VU) is predicted when the level 7 is reached (Sensory level 7 was previously set). The Log10 Y represents a Shelf life ( VU) in days at the selected temperature. For each temperature (room temperature (19-25 º C), 35 ° C and 45 º C) a particular shelf life time (VU) can be predicted for a sensory loss for a7 level ( log of 7 is Log10 Y = 0.8450):

$$VU\ days = t = \frac{(Log_{10} Y) - I}{m}$$ *equation (2)*

Table 3 expresses the shelf life in days for a level 7, where the product has undergone any change in taste, smell and original appearance, without being disagreeable, The greatest loss is highlighted for the attribute which reaches first the detrimental 7 value

***Table 3 Attribute summary relationship for sensory losses by temperature group (Level 7)***

| | AMBIENTE | | | | | | 35ºC | | | | | | 45ºC | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Appearance | Color | Odor | Flavor | Fluidity | Rancidity | Appearance | Color | Odor | Flavor | Fluidity | Rancidity | Appearance | Color | Odor | Flavor | Fluidity | Rancidity |
| **Shelf life VU(7)** | 374 | 374 | 275 | 275 | 275 | 374 | 359 | 151 | 261 | 180 | 250 | 231 | 148 | 75 | 131 | 93 | 109 | 125 |
| **Months** | 12.48 | 12.48 | 9.15 | 9.15 | 9.15 | 12.48 | 11.98 | 5.02 | 8.71 | 6.01 | 8.33 | 7.69 | 4.95 | 2.49 | 4.37 | 3.09 | 3.64 | 4.17 |

Limiting factors are highlighted in red

The interaction of sensory loss (days to reach a deterioration to a level 7) in relation to temperature, is obtained by Equation 3 and fig. 4, the equation that defines these changes is:

Log 10 (decay time) = (m * T) + I equation (3)

m: is the slope
T: is the temperature
I: is the intercept

Integrating the sensory loss for each temperature from table 3 and equation 3, the sensory loss is now associated with temperature (Table 4)

***Table 4. - Projected stability (days) under different temperatures: room temperature (19 - 25 ° C), 35 and 45 º C for a deterioration level of "7".***

| Appearance | | | Color | | | Odor | | | Flavor | | | Fluidity | | | Rancidity | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tem | Days | log10 Days | Tem | Days | log10 Days | Tem | Days | log10 Days | Tem | Days | log10 Days | Tem | Days | log10 Days | Tem | Days | log10 Days |
| 25 | 374 | 2.5733 | 25 | 374 | 2.5733 | 25 | 275 | 2.4387 | 25 | 275 | 2.4387 | 25 | 275 | 2.4387 | 25 | 374 | 2.5733 |
| 35 | 359 | 2.5555 | 35 | 151 | 2.1782 | 35 | 261 | 2.4174 | 35 | 180 | 2.2561 | 35 | 250 | 2.3976 | 35 | 231 | 2.3631 |
| 45 | 148 | 2.1714 | 45 | 75 | 1.8732 | 45 | 131 | 2.1178 | 45 | 93 | 1.9664 | 45 | 109 | 2.0383 | 45 | 125 | 2.0976 |
| m | -0.020 | | m | -0.035 | | m | -0.016 | | m | -0.024 | | m | -0.020 | | m | -0.024 | |
| Int | 3.137 | | Int | 3.433 | | Int | 2.886 | | Int | 3.047 | | Int | 2.992 | | Int | 3.177 | |
| R2 | 0.783 | | R2 | 0.995 | | R2 | 0.800 | | R2 | 0.983 | | R2 | 0.826 | | R2 | 0.996 | |

***Note: Tem = temperature, m = slope, int. = intersection***

***Figure 4. Behavior for a Level of "7": shelf live days (stability expressed as "log days") in relation to temperature***

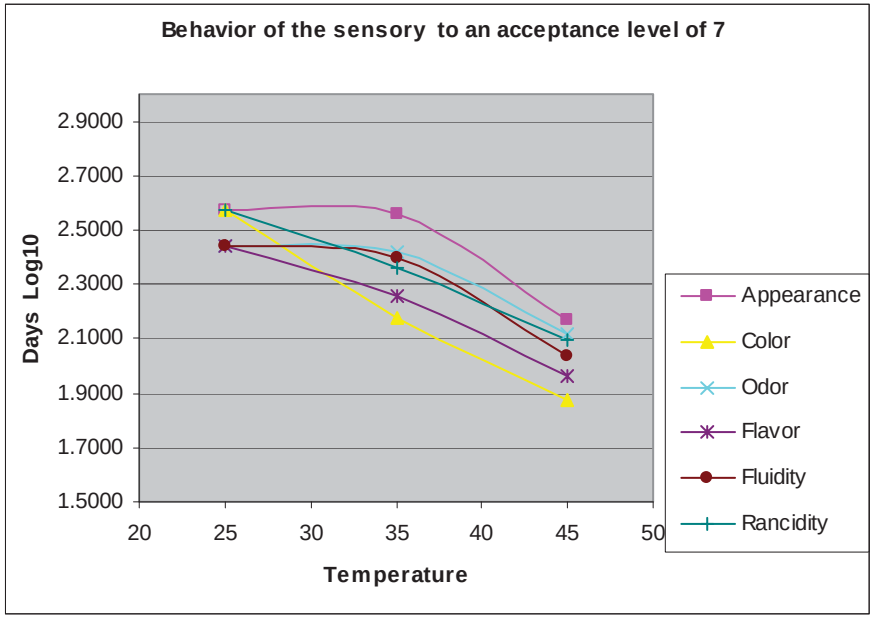
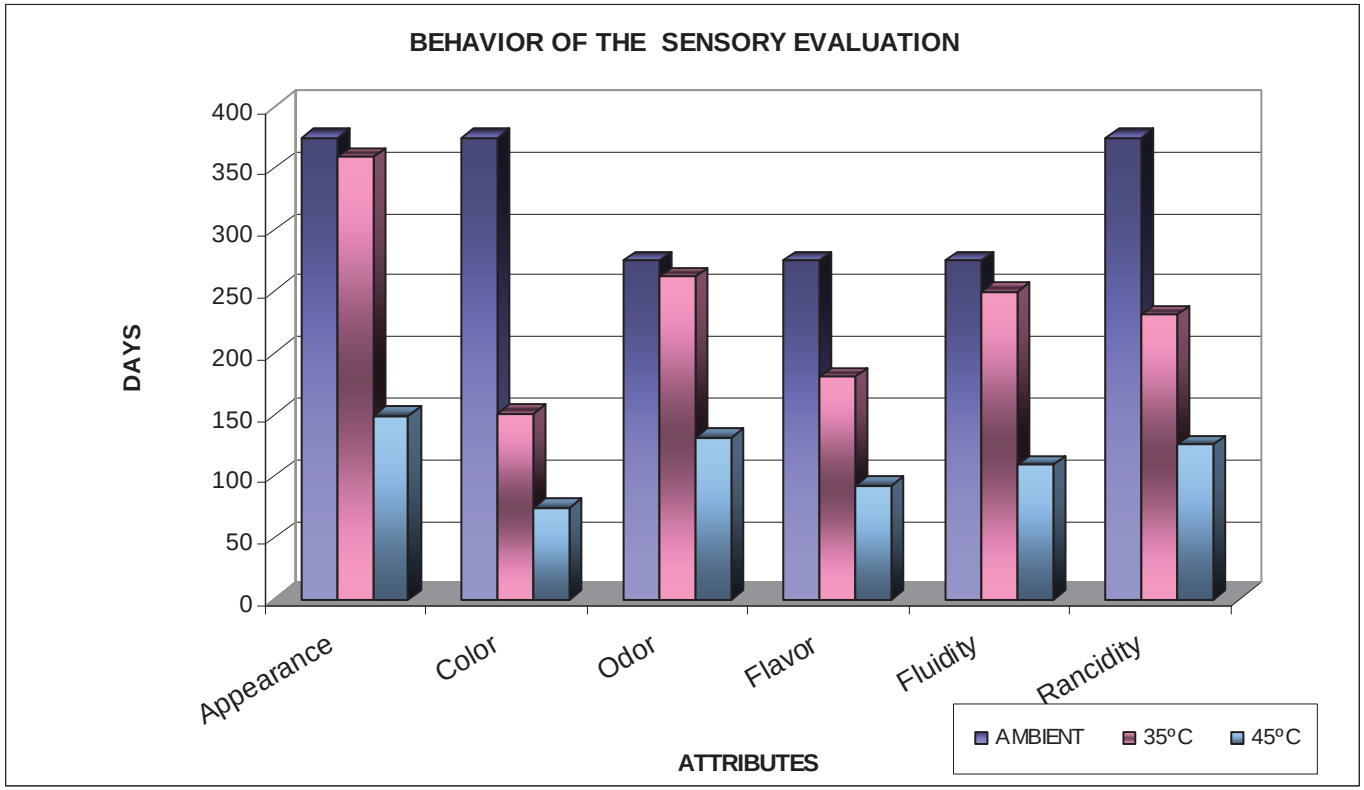
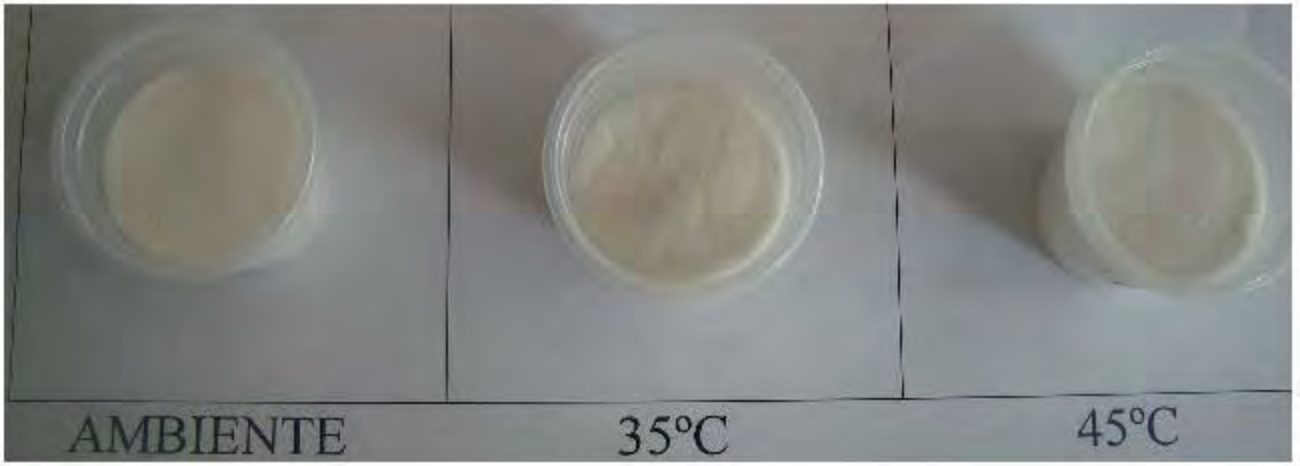


*Table 5 and Figure 5: Projected Days to reach a sensory loss of "7"*

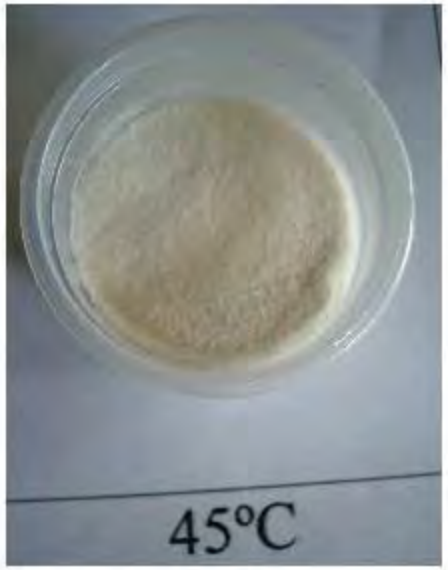
| | Appearance | Color | Odor | Flavor | Fluidity | Rancidity |
|---|---|---|---|---|---|---|
| **Ambient** | 436 | 436 | 508 | 514 | 426 | 504 |
| **35 ºC** | 538 | 150 | 158 | 282 | 274 | 191 |
| 45 ºC | 226 | 393 | 80 | 61 | 116 | 76 |



**PHOTOS:**





### 3.2 Moisture behavior:

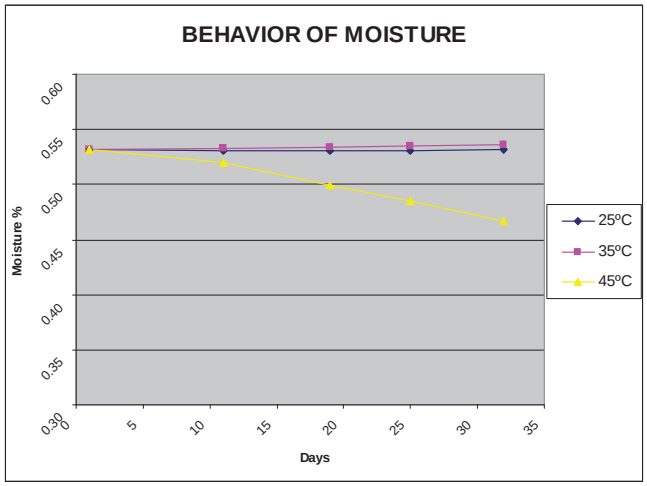
Moisture changes are summarized accordingly to storage time (days) at different temperatures and applying the same sequence of analysis.

***Table 6. Behavior of moisture % about the days at different temperature***

| Moisture % | | | |
|---|---|---|---|
| Days | 25ºC | 35ºC | 45ºC |
| 1 | 3.40 | 3.40 | 3.40 |
| 11 | 3.39 | 3.41 | 3.31 |
| 19 | 3.39 | 3.42 | 3.16 |
| 25 | 3.39 | 3.43 | 3.06 |
| 32 | 3.40 | 3.44 | 2.93 |

| Moisture % | | | |
|---|---|---|---|
| Days | 25ºC | 35ºC | 45ºC |
| 1 | 0.53 | 0.53 | 0.53 |
| 11 | 0.53 | 0.53 | 0.52 |
| 19 | 0.53 | 0.53 | 0.50 |
| 25 | 0.53 | 0.54 | 0.49 |
| 32 | 0.53 | 0.54 | 0.47 |
| m | 0.0000 | 0.0002 | -0.0021 |
| Int | 0.5308 | 0.5311 | 0.5380 |
| R2 | 0.0069 | 0.9907 | 0.9772 |

Figure 7 represents behavior of moisture changes. Its integration with days and temperatures



### 3.3 MICROBIAL CHANGES

**Microbial changes are presented in table 8,**

| MICROBIAL ANALYSIS | | | | |
|---|---|---|---|---|
| | ROOM TEMPERATURE | ROOM TEMPERATURE | 35 ºC | 45ºC |
| | INITIAL | FINAL | FINAL | FINAL |
| Total coliformes | <10 CFU/g | | | |
| E. coli | <3 MPN | | | |
| Yeast | <10 CFU/ g | <10 CFU/g | <10 CFU/g | <10 CFU/g |
| Total plate count | <10 ev CFU/g | <10 CFU/g | <10 CFU/g | <10 CFU/g |
| Molds | <10 CFU/g | <10 CFU/g | <10 CFU/g | <10 CFU/g |
| Salmonella | Absent/ 25g | | | |

**ev= estimated value**

## 4. - CONCLUSIONS

Taking into account the selected scale to estimate changes up to a level 7 (The product has undergone any change in taste, smell and original appearance, without being disagreeable), general considerations are:

The samples at room temperature have a shelf life of 275 days, where the limiting attributes are: odor, flavor and fluidity.

At 35 ° C the estimated shelf life is 151 days; limiting attribute are color and odor.
At 45 ° C a similar change were observed in most of the limiting attributes of: color and flavor, with 75 days of shelf life, followed by taste.

Attributes behave differently at different detrimental speeds; odor, color and flavor are consistently the limiting attributes associated to temperature. Panelists comments are: odor changes and perceived less intense, in particular at 45 º C.

The determination of moisture initial is of 3.4 %, this ratio decreases upon time, final at 2.93 %, at 32 days and a 45 C, Does not affect the fluidity of the product

Microbiological testing implies a stable product.

As summary, the product has a shelf life of 275 days at ambient temperature (19-25 º C), when it reached the sensory value of "7"

**5.0 BIBLIOGRAPHY**


- Taoukis P.S, Labuza T. P, Saguy I.S. 2001. Kinetics of Food Deterioration and shelf life Prediction. The Handbook of Food Engineering Practice. CRC PRESS. Chapter 10.
- Internal method (VU-002-2)



Elaborated by
I.A. Celia Ortigoza Hernández
Sensory Analysis Coordinator

Reviewed by
Dr. Pedro Valle Vega
Technical Director

**10.13** Attachment 13

The iidea Company

Tlaquepaque, Jalisco, México. September 14, 2011

To whom it may concern,

By this mean we assure that the shelf life of Inufib is 3 years. We determined it having as reference other similar products , like powder dextrose.

We protect the product with two polyethylene bags of 200 caliber and three paper kraft bags to avoid humidity absorption.

The results sent by an external laboratory show that the shelf life is less than 3 years, however the methodology used by them was a sensorial analysis. They realized microbiological analysis to the sample and it does not present changes with time.

Based in IIDEA's experience, the chemical composition in the product does not change with time.

Sincerely,

The iidea Company
Premium Agave Quality Products
QUALITY
SSURANCE

[redacted]

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**10.14** Attachment 14

eurofins | analytics

**Sample code Nr.** [redacted] **Date** 07/04/2011 

**Analytical Report Nr.** [redacted]

**Industrializadora Integral del Agave S.A. de C.V.**

For the attention of [redacted]

Periférico Sur 7750
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45601 Tlaquepaque
MEXIQUE

**Email** carolina.santos@iidea.com.mx

**Technical contact for your orders :** Marie Jaillais

**Our reference :** (b) (4) / (b) (4) **Type :** EX
**Client reference :** (b) (4)
**Sample described as :** Organic Agave inulin
INDULINA PREMIUM EN POLVO
**Packaging :** 120g plastic bag
**Sample reception date :** 21/03/2011 **Analysis starting date :** 21/03/2011
**Sampling/Transport :** FedEx
**Analyses requested :** PAG35: PCDD/F (17) + PCB (12) ~ food / feed
PAL1E: Nutritional Labelling - Group EC 1
PAL2A: Nutritional Labelling - Group EC2 complement
J5001: Fructanes : calc. as Inuline
CYP07: dry matter

| Energy values | | | Results (uncertainty) | |
|---|---|---|---|---|
| **AAC99** | **AA** | **Energy values according to EC 2008/100** | | |
| | Energy value (kJ) | | 1313 | kJ/100 g |
| | Energy value (kcal) | | 313 | kcal/100 g |
| **AAC90** | **AA** | **Energy values according to EC 90/496** | | |
| | Energy value (kJ) | | 1014 | kJ/100 g |
| | Energy value (kcal) | | 239 | kcal/100 g |

| Compositional analyses | | | Results (uncertainty) | |
|---|---|---|---|---|
| **AAC00** | **AA** | **Carbohydrate content** | | |
| | Available carbohydrates (by difference) | | 59.7 | g/100 g |
| **A7359** | **AA** | **Moisture oven dry 70°C, Vacuum Method : Arrêté du 8 septembre 1977 adapté** | | |
| | Moisture | | 2.7 | (± 0.5) g/100 g |
| | Total solids | | 97.3 | (± 0.8) g/100 g |
| **AA009** | **AA** | **Ash Method : Arrêté du 8 septembre 1977 adapté** | | |
| | Ash | | 0.37 | (± 0.10) g/100 g |
| **A6201** | **AA** | **Proteins Method : Internal method, Continuous flow** | | |
| | Proteins (Nx6.25) | | <0.5 | g/100 g |
| **AAC08** | **AA** | **Fatty acids in 100 g product calculation** | | |
| | Fatty acids, monounsat. (/product) | | <0.5 | g/100 g |
| | Fatty acids, polyunsat. (/product) | | <0.5 | g/100 g |
| | Fatty acids, saturated. (/product) | | <0.5 | g/100 g |
| | Fatty acids, trans. (/product) | | <0.5 | g/100 g |
| **A6204** | **AA** | **Total fat (acid hydrolysis) Method : AOCS Am 5-04** | | |
| | Fat | | <0.5 | g/100 g |
| **J5001** | **JK** | **Fructanes : calc. as Inuline Method : Internal Method** | | |
| (a) | calculated as Inulin | | 37.3 | g/100 g |
| **A7488** | **AA** | **Sugar profile (IC) Method : Internal method, I.C.** | | |
| | Fructose | | 63.2 | (± 7.0) g/kg |
| | Glucose | | 5.4 | (± 0.9) g/kg |
| | Lactose | | <1.5 | g/kg |
| | Maltose | | <1.5 | g/kg |
| | Maltotriose (IC) | | <1.5 | g/kg |
| | Saccharose | | <1 | g/kg |

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| Compositional analyses | | | Results (uncertainty) | |
|---|---|---|---|---|
| **A7488** | **AA** | **Sugar profile (IC) Method : Internal method, I.C.** | | |
| | Sum of sugars (mono and disaccharides) | | 6.9 | (± 1.3) g/100 g |
| **AA210** | **AA** | **Total Dietary Fiber Method : AOAC 985.29 2003** | | |
| | Fiber content (according to AOAC 985.29) | | <0.5 | g/100 g |

| Minerals - Oligoelements | | | Results (uncertainty) | |
|---|---|---|---|---|
| **AA622** | **AA** | **Sodium Method : Arrêté du 8 septembre 1977 adapté** | | |
| | Sodium (Na) | | 0.0353 | (± 0.0050) g/100 g |

| Fatty acid profile (exp. % total) | | | Results (uncertainty) | |
|---|---|---|---|---|
| **AA251** | **AA** | **Fatty acid composition (GC) Method : EN ISO 15304; EN ISO 5508; EN ISO 5509** | | |
| | Docosadienoic acid C22:2 (n-6) - ω6 | | <0.05 | % |
| | Saturated fatty acids (%total) | | 65.8 | % |
| | Monounsaturated fatty acids (%total) | | 34.2 | % |
| | Polyunsaturated fatty acids (%total) | | <0.05 | % |
| | Total trans-fatty acids (%total) | | <0.05 | % |
| | Not quantifiable fatty acids | | <0.05 | % |
| | Omega-3 fatty acids (%total) | | <0.05 | % |
| | Omega-6 fatty acids (%total) | | <0.05 | % |

| Dioxins | | | Results (uncertainty) | |
|---|---|---|---|---|
| **CYP07** | **GF** | **dry matter Method : DIN 38414-S2** | | |
| (a) | dry residue | | 97.13 | % |
| **A7158** | **GF** | **PCDD/F ~ 17 congeneres ~ food / feed Method : AIR DF 100** | | |
| (a) | 2,3,7,8-TetraCDD | | < 0.01 | ng/kg MC12% |
| (a) | 1,2,3,7,8-PentaCDD | | < 0.01 | ng/kg MC12% |
| (a) | 1,2,3,4,7,8-HexaCDD | | < 0.02 | ng/kg MC12% |
| (a) | 1,2,3,6,7,8-HexaCDD | | < 0.02 | ng/kg MC12% |
| (a) | 1,2,3,7,8,9-HexaCDD | | < 0.02 | ng/kg MC12% |
| (a) | 1,2,3,4,6,7,8-HeptaCDD | | < 0.17 | ng/kg MC12% |
| (a) | OctaCDD | | < 0.55 | ng/kg MC12% |
| (a) | 2,3,7,8-TetraCDF | | < 0.02 | ng/kg MC12% |
| (a) | 1,2,3,7,8-PentaCDF | | < 0.02 | ng/kg MC12% |
| (a) | 2,3,4,7,8-PentaCDF | | < 0.02 | ng/kg MC12% |
| (a) | 1,2,3,4,7,8-HexaCDF | | < 0.02 | ng/kg MC12% |
| (a) | 1,2,3,6,7,8-HexaCDF | | < 0.02 | ng/kg MC12% |
| (a) | 1,2,3,7,8,9-HexaCDF | | < 0.02 | ng/kg MC12% |
| (a) | 2,3,4,6,7,8-HexaCDF | | < 0.02 | ng/kg MC12% |
| (a) | 1,2,3,4,6,7,8-HeptaCDF | | < 0.03 | ng/kg MC12% |
| (a) | 1,2,3,4,7,8,9-HeptaCDF | | < 0.03 | ng/kg MC12% |
| (a) | OctaCDF | | < 0.16 | ng/kg MC12% |
| (a) | WHO(1998)-PCDD/F TEQ excl. LOQ | | ND | ng/kg MC12% |
| (a) | WHO(1998)-PCDD/F-TEQ incl. LOQ | | 0.056 | ng/kg MC12% |

| Dioxin-Like PCBs | | | Results (uncertainty) | |
|---|---|---|---|---|
| **A7347** | **GF** | **PCB ~ dioxin-like / 12 WHO ~ food / feed Method : AIR DF 100** | | |
| (a) | PCB 77 | | < 0.73 | ng/kg MC12% |
| (a) | PCB 81 | | < 0.15 | ng/kg MC12% |
| (a) | PCB 105 | | < 1.52 | ng/kg MC12% |
| (a) | PCB 114 | | < 0.34 | ng/kg MC12% |
| (a) | PCB 118 | | < 5.70 | ng/kg MC12% |
| (a) | PCB 123 | | < 0.46 | ng/kg MC12% |
| (a) | PCB 126 | | < 0.19 | ng/kg MC12% |
| (a) | PCB 156 | | < 1.88 | ng/kg MC12% |
| (a) | PCB 157 | | < 0.33 | ng/kg MC12% |
| (a) | PCB 167 | | < 0.73 | ng/kg MC12% |
| (a) | PCB 169 | | < 0.73 | ng/kg MC12% |
| (a) | PCB 189 | | < 0.48 | ng/kg MC12% |
| (a) | WHO(1998)-PCB-TEQ excl. LOQ | | ND | ng/kg MC12% |
| (a) | WHO(1998)-PCB TEQ incl. LOQ | | 0.029 | ng/kg MC12% |

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| Dioxin-Like PCBs | | | Results (uncertainty) | |
|---|---|---|---|---|
| **GF004** | **GF** | **WHO-PCDD/F+PCB TEQ** | | |
| (a) | WHO(1998)-PCDD/F+PCB TEQ excl. LOQ | | ND | ng/kg MC12% |
| (a) | WHO(1998)-PCDD/F+PCB TEQ incl. LOQ | | 0.085 | ng/kg MC12% |

| Dioxins and PCB TEQ with WHO 2005 TEF | | | Results (uncertainty) | |
|---|---|---|---|---|
| **A7158** | **GF** | **PCDD/F ~ 17 congeneres ~ food / feed Method : AIR DF 100** | | |
| (a) | WHO(2005)-PCDD/F TEQ excl. LOQ | | ND | ng/kg MC12% |
| (a) | WHO(2005)-PCDD/F TEQ incl. LOQ | | 0.052 | ng/kg MC12% |
| **A7347** | **GF** | **PCB ~ dioxin-like / 12 WHO ~ food / feed Method : AIR DF 100** | | |
| (a) | WHO(2005)-PCB TEQ excl. LOQ | | ND | ng/kg MC12% |
| (a) | WHO(2005)-PCB TEQ incl. LOQ | | 0.042 | ng/kg MC12% |
| **GF004** | **GF** | **WHO-PCDD/F+PCB TEQ** | | |
| (a) | WHO(2005)-PCDD/F+PCB TEQ excl. LOQ | | ND | ng/kg MC12% |
| (a) | WHO(2005)-PCDD/F+PCB TEQ incl. LOQ | | 0.094 | ng/kg MC12% |

**SIGNATURE** (b) (6)

Marie Jaillais
alytical Services Manager

Report electronically validated by Marie Jaillais

**EXPLANATORY NOTE**

The analysis are identified by a five-digit code, their description is available on request. $
This document can only be reproduced in full ; it only concerns the submitted sample. Results have been obtained $
and reported in accordance with our general sales conditions available on request. $
In order to state whether the sample complies or not with the specifications of the product, the uncertainty of the result has been taken into account. $

The tests identified by the two letters code JK are performed in laboratory Eurofins Analytik GmbH, Wiertz-Eggert-Jörissen. The symbol (a) identified the tests performed under accreditation DIN EN ISO/IEC 17025:2005 D-PL-14251-01-00 $
The tests identified by the two letters code GF are performed in laboratory Eurofins GfA Gmbh Hamburg. The symbol (a) identified the tests performed under accreditation DIN EN ISO/IEC 17025:2005 D-PL-14199-01-00 $
The tests identified by the two letters code AA are performed in laboratory Eurofins Analytics France. $

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**At'n:** [redacted]

**REF (S.A.)** [redacted]
**Arrival date:** 18/03/11
**Analysis date:** 22/03/11
**Deliver date:** 03/05/11

## ANALYSIS REPORT

**SAMPLE (S) DESCRIPTION** *Sample(s) received in this lab for its study*

**1.- AGAVE INULIN POWDER,** CODE NUMBER: [redacted] MATRIX SAMPLE: RAW MATERIAL

**I.- ATOMIC ABSORPTION ANALYSIS**

| Analysis | Result (s) | Method |
|---|---|---|
| Arsenic (mcg/kg) | Not detected | NOM-117-SSA1-1994 |
| Cadmium (mg/kg) | Not detected | NOM-117-SSA1-1994 |
| Mercury mcg/kg) | Not detected | NOM-117-SSA1-1994 |
| Lead (mg/kg) | Not detected | NOM-117-SSA1-1994 |

[redacted]

*I.Q.P. Fernando Cruz Cortés*
*Chemistry Supervisor*

[redacted]

*I.Q. Fabián A. Gómez Martínez*
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## ANALYSIS REPORT

**SAMPLE (S) DESCRIPTION** *Sample(s) received in this lab for its study*

**1.- AGAVE INULIN POWDER,** CODE NUMBER: [redacted], MATRIX SAMPLE: RAW MATERIAL

**I.- GAS CROMATOGRAPHY ANALYSIS**

| Analysis | Result (s) | Analysis | Result (s) | Method |
|---|---|---|---|---|
| 3-keto Carbofuran | Not detected | Butachlor | Not detected | Pesticide Analytical Manual Vol. 1 |
| Aldicarb | Not detected | Captafol (Difolatan) | Not detected | |
| Aldicarb Sulfone | Not detected | Captan | Not detected | |
| Alidicarb sulfoxide | Not detected | Carfentrazone-ethyl | Not detected | |
| Aminocarb | Not detected | Clordane | Not detected | |
| Bendiocarb | Not detected | Cyanazine | Not detected | |
| Bufencarb | Not detected | Cyfluthrin | Not detected | |
| Carbaryl | Not detected | Cypermethrin | Not detected | |
| Carbofuran | Not detected | Cyproconazole | Not detected | |
| Ethiofencarb | Not detected | Chlordane | Not detected | |
| Fenobucarb | Not detected | Chlordimeform | Not detected | |
| Methiocarb | Not detected | Chlorfenapyr | Not detected | |
| Methiocarb sulfone | Not detected | Chlorfenson | Not detected | |
| Methiocarb sulfoxide | Not detected | Chlornitrofen | Not detected | |
| Methomyl | Not detected | Chlorobenzilate | Not detected | |
| Oxamyl | Not detected | Chloroneb | Not detected | |
| Propoxur | Not detected | Chlorothalonil | Not detected | |
| Thiodicarb | Not detected | Chloroxuron | Not detected | |
| Acetamiprid | Not detected | Chlorpropham | Not detected | |
| Alachlor | Not detected | Dactal (DCPA) | Not detected | |
| Aldrin | Not detected | d-BHC (d-HCH) | Not detected | |
| alpha-BCH- (a-HCH) | Not detected | DCPA (Dacthal) | Not detected | |
| Anilazine | Not detected | DDD | Not detected | |
| b-BHC (b-HCH) | Not detected | DDE | Not detected | |
| Benzoylprop-ethyl | Not detected | DDT | Not detected | |
| BHC, alpha | Not detected | Deltamethrin | Not detected | |
| BHC, beta | Not detected | Diclobutrazol | Not detected | |
| BHC, delta | Not detected | Dicofol | Not detected | |
| Bifenox | Not detected | Dichlobenil | Not detected | |
| Bifenthrin | Not detected | Dichlofluanid | Not detected | |
| Boscalid | Not detected | Dichlone | Not detected | |
| Bromacil | Not detected | Dieldrin | Not detected | |

[redacted]
*I.Q.P. Fernando Cruz Cortés*
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[redacted]
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## ANALYSIS REPORT

**SAMPLE (S) DESCRIPTION** *Sample(s) received in this lab for its study*

**1.- AGAVE INULIN POWDER,** CODE NUMBER: [redacted] MATRIX SAMPLE: RAW MATERIAL

**I.- GAS CROMATOGRAPHY ANALYSIS**

| Analysis | Result (s) | Analysis | Result (s) | Method |
|---|---|---|---|---|
| Diethatyl-ethyl | Not detected | Mirex | Not detected | Pesticide Analytical Manual Vol. 1 |
| Dimethachlor | Not detected | Monolinuron | Not detected | |
| Dimethomorph | Not detected | Myclobutanil | Not detected | |
| Endosulfan I | Not detected | Nitrofen | Not detected | |
| Endosulfan II | Not detected | Nuarimol | Not detected | |
| Endosulfan Sulfate | Not detected | Ofurace | Not detected | |
| Endrin | Not detected | Oxadiazon | Not detected | |
| Esfenvalerate | Not detected | Oxadixyl | Not detected | |
| Etaconazole | Not detected | Oxyfluorfen | Not detected | |
| Ethylan | Not detected | Paclobutrazol | Not detected | |
| Fenarimol | Not detected | PCNB (Quintozene) | Not detected | |
| Fenazquin | Not detected | Penconazole | Not detected | |
| Fenbuconazole | Not detected | Pentachloroaniline | Not detected | |
| Fenhexamid | Not detected | Permethrin | Not detected | |
| Fenson | Not detected | Perthane | Not detected | |
| Fenvalerate | Not detected | PP-DDE | Not detected | |
| Flucythrinate | Not detected | PP-DDT | Not detected | |
| Folpet | Not detected | Procymidone | Not detected | |
| Fuchloralin | Not detected | Prochloraz | Not detected | |
| Hepta epóxido | Not detected | Pronamide | Not detected | |
| Heptachlor | Not detected | Propachlor | Not detected | |
| Heptachlor epoxide | Not detected | Propanil | Not detected | |
| Hexaconazole | Not detected | Propiconazole | Not detected | |
| Hexachlorobenzene | Not detected | Pyraclostrobin | Not detected | |
| Imazalil | Not detected | Pyridaben | Not detected | |
| Indoxacarb | Not detected | Pyrifenox | Not detected | |
| Iprodione | Not detected | Quinoxyfen | Not detected | |
| Lambda-Cyhalothrin | Not detected | Simazine | Not detected | |
| Lindane | Not detected | Simetryn | Not detected | |
| Linuron | Not detected | Tebuconazole | Not detected | |
| Methoxychlor | Not detected | Terbacil | Not detected | |
| Metolachlor | Not detected | | | |

[redacted]

*I.Q.P. Fernando Cruz Cortés*
*Chemistry Supervisor*

[redacted]

*I.Q. Fabián A. Gómez Martínez*
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**At'n:** [redacted]

**REF (S.A.)** [redacted]
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**Deliver date:** 03/05/11

## ANALYSIS REPORT

**SAMPLE (S) DESCRIPTION** *Sample(s) received in this lab for its study*

**1.- AGAVE INULIN POWDER,** CODE NUMBER: [redacted] MATRIX SAMPLE: RAW MATERIAL

**I.- GAS CROMATOGRAPHY ANALYSIS**

| Analysis | Result (s) | Analysis | Result (s) | Method |
|---|---|---|---|---|
| Tetradifon | Not detected | Diazinon | Not detected | |
| Thiobencarb | Not detected | Dicrotophos | Not detected | |
| Thiodan I | Not detected | Dichlorvos / DDVP Vapona | Not detected | |
| Thiodan II | Not detected | Dimethoate | Not detected | |
| Tolylfluanid | Not detected | Dioxathion | Not detected | |
| Toxaphene | Not detected | Disulfoton (Dy-siston) | Not detected | |
| Tralomethrin | Not detected | Disulfoton-sulfone | Not detected | |
| Triadimenol | Not detected | Edifenphos | Not detected | |
| Triflumizole | Not detected | Ethion (Nialate) | Not detected | |
| Vegadex | Not detected | Etrimfos | Not detected | |
| Vinclozolin | Not detected | Fenamiphos | Not detected | |
| Acephate | Not detected | Fenitrothion | Not detected | |
| Azinphos-ethyl | Not detected | Fensulfothion | Not detected | |
| Azinphos-methyl | Not detected | Fenthion | Not detected | |
| Bensulide | Not detected | Fonofos | Not detected | |
| Bromophos | Not detected | Forate (Thimet) | Not detected | Pesticide Analytical Manual Vol. 1 |
| Bromophos-ethyl | Not detected | Formothion | Not detected | |
| Cadusafos | Not detected | Guthion | Not detected | |
| Carbophenothion OA | Not detected | Heptenophos | Not detected | |
| Carbophenotion | Not detected | Iprobenfos | Not detected | |
| Coumaphos | Not detected | Isazophos | Not detected | |
| Crotoxyphos | Not detected | Isofenphos | Not detected | |
| Cyanophos | Not detected | Leptophos | Not detected | |
| Chlorfenvinphos | Not detected | Malaoxon | Not detected | |
| Chlorpyrifos | Not detected | Malathion (Cithion) | Not detected | |
| Chlorpyrifos-methyl | Not detected | Metasystox-R | Not detected | |
| Chlorthiophos | Not detected | Methacrifos | Not detected | |
| DEF | Not detected | Methamidophos | Not detected | |
| Demeton-o | Not detected | Methidathion | Not detected | |
| Demeton-s | Not detected | Methyl Trithion | Not detected | |
| Demeton-s-sulfone | Not detected | Mevinfos | Not detected | |
| Dialifos | Not detected | Mimethoate | Not detected | |

[redacted]

*I.Q.P. Fernando Cruz Cortés*
*Chemistry Supervisor*

[redacted]

*I.Q. Fabián A. Gómez Martínez*
*Laboratory Director*

FGM/FCC/BSCM

Any commentary or complains about our service please report it to fax 26-14-11-42 to the Attention of the Laboratory Director
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SILLIKER
a Mérieux NutriSciences Company

AC-F-007-3 Mexico City Unit

**Company:** **INDUSTRIALIZADORA INTEGRAL DEL AGAVE, S.A. DE C.V.**
**Adress:** Periférico Sur 7750, Santa Maria Tequepexpan, Tlaquepaque Jalisco, C.P. 45601
**At'n:** [redacted]

**REF (S.A.)** [redacted]
**Arrival date:** 18/03/11
**Analysis date:** 22/03/11
**Deliver date:** 03/05/11

## ANALYSIS REPORT

**SAMPLE (S) DESCRIPTION** *Sample(s) received in this lab for its study*

**1.- AGAVE INULIN POWDER,** CODE NUMBER: [redacted] MATRIX SAMPLE: RAW MATERIAL

**I.- GAS CROMATOGRAPHY ANALYSIS**

| Analysis | Result (s) | Analysis | Result (s) | Method |
|---|---|---|---|---|
| Monocrotophos | Not detected | Ametryn | Not detected | |
| M-parathion | Not detected | Amitraz | Not detected | |
| Naled | Not detected | Atrazine | Not detected | |
| Omethoate | Not detected | Azoxystrobin | Not detected | |
| Oxidemeton Methyl | Not detected | Benalaxyl | Not detected | |
| Parathion | Not detected | Benefin | Not detected | |
| Parathion-methyl | Not detected | Benfluralin | Not detected | |
| Phenthoate | Not detected | Biphenyl | Not detected | |
| Phorate | Not detected | Bitertanol | Not detected | |
| Phosalone | Not detected | Bromopropylate | Not detected | |
| Phosfolan | Not detected | Bupirimate | Not detected | |
| Phosmet | Not detected | Buprofezin | Not detected | |
| Phosphamidon | Not detected | Carbetamide | Not detected | |
| Piperophos | Not detected | Carbosulfan | Not detected | |
| Pirimiphos | Not detected | Cycloate | Not detected | |
| Pirimiphos-methyl | Not detected | Cyprodinil | Not detected | Pesticide Analytical Manual Vol. 1 |
| Propetamphos | Not detected | Chlorfenapyr | Not detected | |
| Prothiofos | Not detected | Chlozolinate | Not detected | |
| Prothoate | Not detected | Dimethametryn | Not detected | |
| Pyrazophos | Not detected | Dimetoato (Cygon) | Not detected | |
| Pyridaphenthion | Not detected | Diphenylamine | Not detected | |
| Quinalphos | Not detected | EPN | Not detected | |
| Ronnel | Not detected | EPTC | Not detected | |
| Sulprofos | Not detected | Ethalfluralin | Not detected | |
| Terbufos | Not detected | Ethirimol | Not detected | |
| Tetrachlorvinphos | Not detected | Ethofumesate | Not detected | |
| Thiometon | Not detected | Ethoprop | Not detected | |
| Thionazin | Not detected | Ethoxyquin | Not detected | |
| Toclofos-methyl | Not detected | Fenamidone | Not detected | |
| Triazophos | Not detected | Fenpropathrin | Not detected | |
| Acrinathrin | Not detected | Fenpropimorph | Not detected | |
| Ametrine | Not detected | Fipronil | Not detected | |

[redacted]
*I.Q.P. Fernando Cruz Cortés*
*Chemistry Supervisor*

[redacted]
*I.Q. Fabián A. Gómez Martínez*
*Laboratory Director*

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000152

a Mérieux NutriSciences Company

AC-F-007-3 Mexico City Unit

**Company:** **INDUSTRIALIZADORA INTEGRAL DEL AGAVE, S.A. DE C.V.**
**Adress:** Periférico Sur 7750, Santa María Tequepexpan, Tlaquepaque Jalisco, C.P. 45601
**At'n:** [redacted]

**REF (S.A.)** [redacted]
**Arrival date:** 18/03/11
**Analysis date:** 22/03/11
**Deliver date:** 03/05/11

## ANALYSIS REPORT

**SAMPLE (S) DESCRIPTION** *Sample(s) received in this lab for its study*

**1.- AGAVE INULIN POWDER,** CODE NUMBER: [redacted] MATRIX SAMPLE: RAW MATERIAL

**I.- GAS CROMATOGRAPHY ANALYSIS**

| Analysis | Result (s) | Analysis | Result (s) | Method |
|---|---|---|---|---|
| Fludioxonil | Not detected | Promecarb | Not detected | Pesticide Analytical Manual Vol. 1 |
| Flurochloridone | Not detected | Prometon | Not detected | |
| Fluzilazole | Not detected | Prometryn | Not detected | |
| Furalaxyl | Not detected | Propargite | Not detected | |
| Iprodione | Not detected | Propham | Not detected | |
| Kresoxim-methyl | Not detected | Pymetrozine | Not detected | |
| Linuron | Not detected | Pyrethrins | Not detected | |
| Mecarbam | Not detected | Pyrifenox | Not detected | |
| Metalaxyl-M (Mefenoxam) | Not detected | Pyrimethanil | Not detected | |
| Metazachlor | Not detected | Pyriproxyfen | Not detected | |
| Methoprotryn | Not detected | Quinomethionate | Not detected | |
| Metribuzin | Not detected | Tebufenpyrad | Not detected | |
| Mexacarbate | Not detected | Tecnazene | Not detected | |
| Napropamine (Devrinol) | Not detected | Tefluthrin | Not detected | |
| Norflurazon | Not detected | Thiabendazole | Not detected | |
| o-Phenylphenol | Not detected | Tolyfluanid | Not detected | |
| Oryzalin | Not detected | Triadimefon | Not detected | |
| Pendimethalin | Not detected | Trifloxystrobin | Not detected | |
| Pirimicarb | Not detected | Trifluralin | Not detected | |
| Profluralin | Not detected | | | |

[redacted]

*I.Q.P. Fernando Cruz Cortés*
*Chemistry Supervisor*

[redacted]

*I.Q. Fabián A. Gómez Martínez*
*Laboratory Director*

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**10.15** Attachment 15

## Methods of Analysis

We hereby confirm the methods of analysis and the official references used in the Agave Inulin production process:

Internal Analyses:

| *Assay* | *IIDEA Document Number* | *Official Reference* |
|---|---|---|
| FRUCTOSE | MALB-02 | NMX-FF-110-SCFI-2008 |
| GLUCOSE | MALB-02 | NMX-FF-110-SCFI-2008 |
| SUCROSE | MALB-02 | NMX-FF-110-SCFI-2008 |
| INULIN | MALB-02 | NMX-FF-110-SCFI-2008 |
| OTHER CARBOHYDRATES | MALB-02 | NMX-FF-110-SCFI-2008 |
| TOTAL COUNT OF MESOPHYLIC AEROBIC MICROORGANISMS | MALB-02 | NOM-092-SSA1-1994 |
| COLIFORM MICROORGANISMS | MALB-02 | NOM-112-SSA1-1994 |
| YEAST AND MOLDS | MALB-02 | NOM-111-SSA1-1994 |
| HUMIDITY | MALB-02 | NMX-F-591-SCFI-2010 |
| pH | MALB-02 | NMX-F-317-S-1978 |
| FOREIGN MATTER | MALB-02 | NMX-F-591-SCFI-2010 |
| ASHES (% dry matter) | MALB-02 | NMX-F-607-NORMEX |
| TOTAL REDUCING SUGARS (%) | MALB-02 | NMX-V-006-NORMEX-2005 |
| DEGREES BRIX | MALB-02 | NMX-F-103-NORMEX-2009 |

External Analyses:

| *Assay* | *Official Reference* |
|---|---|
| SALMONELLA | NOM-114-SSA1-1994 |
| HEAVY METALS | NOM-117-SSA1-1997 |

[redacted]

*Quality Assurance*

**10.16** Attachment 16

The Idea Company
Premium Agave Products

## AGAVE INULIN INTENDED USES

Agave Inulin is a prebiotic ingredient that belongs to a class of fiber known as fructans. Agave Inulin is an organic dietary fiber which is extracted from the *Tequilana* Weber *Agave* plant. A prebiotic is a non-digestible food ingredient that beneficially affects the body by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon to improve body health. Agave Inulin is not digested in the upper gastrointestinal tract, resulting in reduced caloric value and will not lead to a rise in serum glucose or stimulate insulin secretion. In addition, Agave Inulin aids to increase calcium and magnesium absorption. Agave Inulin has a neutral, sweet clean flavor and is used to improve the mouth feel, stability and acceptability of low fat foods. It can be used to fortify foods with fiber and to improve the flavor and sweetness of low calorie foods. Agave Inulin also improves the texture of fat-reduced foods. Agave Inulin with its high solubility in cold water, can easily be incorporated into beverages, bakery products, and dairy products. Agave Inulin has a unique ability to add textural properties to food. Inulin gels are very creamy and fat-like, and as such can be used as a bulking agent and in fat reduction and fat replacement. Agave Inulin also serves as a source of reduced energy carbohydrates for use as a sugar replacer.

Agave Inulin is proposed for addition to various foods categories as specified in the next table:

| Food Category |
|---|
| Bakery and baked goods |
| Sauces and gravies |
| Ready-to-eat cereals |
| Juice and juice drink beverages |
| Condiments |
| Dairy products |
| Chocolates |
| Protein Drugs |
| Nutritional supplement products |

Other intended uses of Agave Inulin, include the following

| Other intended uses |
|---|
| Personal care products |
| Body care products |

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**10.17** Attachment 17

| Table A. Estimated Daily Intake of Inufib™ and Inulin from Proposed Uses by U.S. Infants Under 1 Year of Age | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Users | | 2-day Average Inufib™ Intake (g/d) | | 2-Day Average Inulin Intake from Dry Inufib™ (g/d) | | 2-Day Average Inulin Intake from Liquid Inufib™ (g/d) | |
| Food Category | N | % | Mean | 90th Percentile | Mean | 90th Percentile | Mean | |
| ALL CATEGORIES COMBINED | 849 | 80.3 | 1.3 | 2.6 | 1.1 | 2.3 | | |
| Baby foods | 0 | – | – | – | – | – | | |
| Baked goods, lite cakes | 1 | 0.1 | 0.7 | 0.7 | 0.6 | 0.6 | | |
| Baked goods, lite cookies | 1 | 0.1 | 3.5 | 3.5 | 3.1 | 3.1 | | |
| Bars | 4 | 0.5 | 1.4 | 3.3 | 1.2 | 2.9 | | |
| Beverages, fermented milks | 0 | – | – | – | – | – | | |
| Beverages, functional | 1 | <0.05 | 29.1 | 29.1 | 25.6 | 25.6 | 23.3 | |
| Beverages, juices and juice drinks | 237 | 22.9 | 2.5 | 4.7 | 2.2 | 4.1 | | |
| Beverages, milk-based | 7 | 0.4 | 0.8 | 1.9 | 0.7 | 1.7 | | |
| Biscuits, reduced fat | 1 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | | |
| Breads, conventional | 195 | 18.0 | 0.1 | 0.2 | 0.1 | 0.2 | | |
| Breads, specialty | 3 | 0.3 | 0.4 | 0.9 | 0.4 | 0.8 | | |
| Candy, hard dietetic | 0 | – | – | – | – | – | | |
| Candy, soft dietetic | 0 | – | – | – | – | – | | |
| Condiments | 12 | 1.3 | 0.1 | 0.3 | 0.1 | 0.3 | | |
| Cream cheese, reduced fat | 0 | – | – | – | – | – | | |
| French fry coatings | 58 | 5.8 | 0.3 | 0.4 | 0.3 | 0.4 | | |
| Frozen dairy desserts, lite | 4 | 0.3 | 2.5 | 3.5 | 2.2 | 3.1 | | |
| Icings/glazes, lite | 0 | – | – | – | – | – | | |
| Jams and jellies, lite | 1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | | |
| Meat products | 0 | – | – | – | – | – | | |
| Mousse, reduced fat[c] | 0 | – | – | – | – | – | | |

**Table A. (cont.) Estimated Daily Intake of Inufib™ and Inulin from Proposed Uses by U.S. Infants Under 1 Year of Age**

| | Users | | 2-day Average Inufib™ Intake (g/d) | | 2-Day Average Inulin Intake from Dry Inufib™ (g/d) | | 2-Day Average Inulin Intake from Liquid Inufib™ (g/d) | |
|---|---|---|---|---|---|---|---|---|
| **Food Category** | **N** | **%** | **Mean** | **90th Percentile** | **Mean** | **90th Percentile** | **Mean** | |
| Pancake syrup, lite | 3 | 0.3 | 0.1 | 0.1 | 0.1 | 0.1 | | |
| Pasta fillings | 8 | 0.7 | 0.4 | 0.7 | 0.4 | 0.6 | | |
| Pasta, fresh[d] | 48 | 4.7 | 0.7 | 1.4 | 0.6 | 1.2 | | |
| Pasta, precooked macaroni[d] | 46 | 4.2 | 1.1 | 2 | 1.0 | 1.8 | | |
| Pizza crust | 24 | 2.7 | 0.4 | 0.6 | 0.4 | 0.5 | | |
| Potatoes, mashed | 63 | 6.5 | 1.3 | 3.2 | 1.1 | 2.8 | | |
| Pretzels, soft | 1 | 0.1 | 1.4 | 1.4 | 1.2 | 1.2 | | |
| Processed cheese, reduced fat | 1 | 0.1 | 0.5 | 0.5 | 0.4 | 0.4 | | |
| Pudding mix | 10 | 1.2 | 0.5 | 0.8 | 0.4 | 0.7 | | |
| RTE breakfast cereals | 128 | 12.9 | 1.5 | 3.1 | 1.3 | 2.7 | | |
| Salad dressings, lite | 0 | – | – | – | – | – | | |
| Sauces and gravies | 189 | 18.1 | 0.7 | 1.7 | 0.6 | 1.5 | | |
| Snack chips, reduced fat | 0 | – | – | – | – | – | | |
| Snack crackers | 77 | 6.9 | 0.3 | 0.6 | 0.3 | 0.5 | | |
| Soups, dry | 25 | 2.0 | 0.4 | 1 | 0.4 | 0.9 | | |
| Spreads, reduced fat | 41 | 4.0 | 0.3 | 0.7 | 0.3 | 0.6 | | |
| Surimi | 0 | – | – | – | – | – | | |
| Toppings, dessert | 2 | 0.3 | 0.1 | 0.1 | 0.1 | 0.1 | | |
| Tortillas, reduced fat[c] | 26 | 2.3 | 0.2 | 0.4 | 0.2 | 0.4 | | |
| Vegetarian patties/crumbles | 1 | 0.1 | 0.4 | 0.4 | 0.4 | 0.4 | | |
| Pancake syrup, lite | 3 | 0.3 | 0.1 | 0.1 | 0.1 | 0.1 | | |
| Pasta fillings | 8 | 0.7 | 0.4 | 0.7 | 0.4 | 0.6 | | |

| Table A. (cont.) Estimated Daily Intake of Inufib™ and Inulin from Proposed Uses by U.S. Infants Under 1 Year of Age | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Users | | 2-day Average Inufib™ Intake (g/d) | | 2-Day Average Inulin Intake from Dry Inufib™ (g/d) | | 2-Day Average Inulin Intake from Liquid Inufib™ (g/d) | |
| Food Category | N | % | Mean | 90th Percentile | Mean | 90th Percentile | Mean | |
| Whipped toppings, lite | 1 | 0.1 | 1.3 | 1.3 | 1.1 | 1.1 | | |
| Yogurt, reduced fat | 36 | 3.9 | 2.7 | 5.5 | 2.4 | 4.8 | | |

Data source: USDA Continuing Survey of Food Intakes by Individuals (CSFII), 1994-96, 1998. Breastfeeding infants and children w from the analysis. Estimates are based on food consumption reported by individuals who provided two 24-hour diet recalls and ma use levels consistent with maximum Frutifit use levels specified in FDA GRN # 118.

a) Dry Inufib™ is 88% inulin by weight.
b) Liquid Inufib™ is 80% inulin by weight.
c) No food codes for reduced fat forms of this food category are in the 1994-96, 98 CSFII; estimates are based on consum versions.
d) No food codes for fresh pasta or precooked macaroni are in the 1994-96, 98 CSFII; estimates are based on consum macaroni.

Note: Unless indicated otherwise, all food categories include both regular and lite versions of all food products.

| Table B. Estimated Daily Intake of Inufib™ and Inulin from Proposed Uses by U.S. Infants 1 Year of Age | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Users | | 2-day Average Inufib™ Intake (g/d) | | 2-Day Average Inulin Intake from Dry Inufib™ (g/d) | | 2-Day Average Inulin Intake from Liquid Inufib™ (g/d) | |
| Food Category | N | % | Mean | 90th Percentile | Mean | 90th Percentile | Mean | |
| ALL CATEGORIES COMBINED | 967 | 99.6 | 7.37 | 15 | 6.5 | 13.2 | | |
| Baby foods | 0 | – | – | – | – | – | | |
| Baked goods, lite cakes | 9 | 0.7 | 0.6 | 1.3 | 0.5 | 1.1 | | |
| Baked goods, lite cookies | 15 | 1.6 | 1 | 1.4 | 0.9 | 1.3 | | |
| Bars | 47 | 5.4 | 2 | 2.8 | 1.8 | 2.5 | | |
| Beverages, fermented milks | 0 | – | – | – | – | – | | |
| Beverages, functional | 4 | 0.4 | 7.6 | 14.0 | 6.7 | 12.3 | | |
| Beverages, juices and juice drinks | 727 | 75.3 | 4 | 7.9 | 3.5 | 7.0 | | |
| Beverages, milk-based | 65 | 6.1 | 1.5 | 3.8 | 1.3 | 3.3 | | |
| Biscuits, reduced fat | 1 | 0.1 | 0.9 | 0.9 | 0.8 | 0.8 | | |
| Breads, conventional | 760 | 78 | 0.1 | 0.3 | 0.1 | 0.3 | | |
| Breads, specialty | 14 | 1.3 | 0.6 | 1.0 | 0.5 | 0.9 | | |
| Candy, hard dietetic | 0 | – | – | – | – | – | | |
| Candy, soft dietetic | 0 | – | – | – | – | – | | |
| Condiments | 240 | 24.2 | 0.3 | 0.7 | 0.3 | 0.6 | | |
| Cream cheese, reduced fat | 5 | 0.4 | 0.2 | 0.4 | 0.2 | 0.4 | | |
| French fry coatings | 253 | 25.8 | 0.4 | 0.9 | 0.4 | 0.8 | | |
| Frozen dairy desserts, lite | 35 | 2.9 | 3.4 | 7 | 3.0 | 6.2 | | |
| Icings/glazes, lite | 1 | 0.1 | 0.1 | 0.1 | < 0.05 | < 0.05 | < 0.05 | |
| Jams and jellies, lite | 10 | 0.9 | 0.1 | 0.2 | 0.1 | 0.2 | | |
| Meat products | 0 | – | – | – | – | – | | |
| Mousse, reduced fat[c] | 0 | – | – | – | – | – | | |

| Table B. (cont.) Estimated Daily Intake of Inufib™ and Inulin from Proposed Uses by U.S. Infants 1 Year of Age | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Users | | 2-day Average Inufib™ Intake (g/d) | | 2-Day Average Inulin Intake from Dry Inufib™ (g/d) | | 2-Day Average Inulin Intake from Liquid Inufib™ (g/d) | |
| Food Category | N | % | Mean | 90th Percentile | Mean | 90th Percentile | Mean | |
| Pancake syrup, lite | 26 | 3.2 | 0.2 | 0.5 | 0.2 | 0.4 | | |
| Pasta fillings | 46 | 4.7 | 0.6 | 1.1 | 0.5 | 1.0 | | |
| Pasta, fresh[d] | 213 | 22.0 | 0.8 | 1.5 | 0.7 | 1.3 | | |
| Pasta, precooked macaroni[d] | 152 | 16.2 | 1.5 | 3.4 | 1.3 | 3.0 | | |
| Pizza crust | 160 | 17.5 | 0.7 | 1.2 | 0.6 | 1.1 | | |
| Potatoes, mashed | 128 | 12.6 | 1.4 | 3.2 | 1.2 | 2.8 | | |
| Pretzels, soft | 8 | 0.7 | 1.5 | 2.5 | 1.3 | 2.2 | | |
| Processed cheese, reduced fat | 7 | 0.7 | 0.4 | 0.7 | 0.4 | 0.6 | | |
| Pudding mix | 30 | 3.0 | 0.6 | 1.0 | 0.5 | 0.9 | | |
| RTE breakfast cereals | 598 | 62.1 | 2.7 | 5.5 | 2.4 | 4.8 | | |
| Salad dressings, lite | 21 | 2.0 | 0.2 | 0.4 | 0.2 | 0.4 | | |
| Sauces and gravies | 686 | 71.4 | 1 | 2.2 | 0.9 | 1.9 | | |
| Snack chips, reduced fat | 9 | 1.0 | 0.2 | 0.3 | 0.2 | 0.3 | | |
| Snack crackers | 245 | 26.5 | 0.4 | 1 | 0.4 | 0.9 | | |
| Soups, dry | 72 | 7.7 | 0.5 | 0.8 | 0.4 | 0.7 | | |
| Spreads, reduced fat | 213 | 21.5 | 0.3 | 0.6 | 0.3 | 0.5 | | |
| Surimi | 1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | | |
| Toppings, dessert | 7 | 0.7 | 0.1 | 0.4 | 0.1 | 0.4 | | |
| Tortillas, reduced fat[c] | 103 | 11.1 | 0.4 | 0.7 | 0.4 | 0.6 | | |
| Vegetarian patties/crumbles | 3 | 0.3 | 0.4 | 0.6 | 0.4 | 0.5 | | |
| Pancake syrup, lite | 26 | 3.2 | 0.2 | 0.5 | 0.2 | 0.4 | | |
| Pasta fillings | 46 | 4.7 | 0.6 | 1.1 | 0.5 | 1.0 | | |

| Table B. (cont.) Estimated Daily Intake of Inufib™ and Inulin from Proposed Uses by U.S. Infants 1 Year of Age | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Users | | 2-day Average Inufib™ Intake (g/d) | | 2-Day Average Inulin Intake from Dry Inufib™ (g/d) | | 2-Day Average Inulin Intake from Liquid Inufib™ (g/d) | |
| Food Category | N | % | Mean | 90th Percentile | Mean | 90th Percentile | Mean | |
| Whipped toppings, lite | 0 | – | – | – | – | – | | |
| Yogurt, reduced fat | 114 | 11.8 | 2.6 | 5.1 | 2.3 | 4.5 | | |

Data source: USDA Continuing Survey of Food Intakes by Individuals (CSFII), 1994-96, 1998. Breastfeeding infants and children w from the analysis. Estimates are based on food consumption reported by individuals who provided two 24-hour diet recalls and ma use levels consistent with maximum Frutifit use levels specified in FDA GRN # 118.

a) Dry Inufib™ is 88% inulin by weight.
b) Liquid Inufib™ is 80% inulin by weight.
c) No food codes for reduced fat forms of this food category are in the 1994-96, 98 CSFII; estimates are based on consum versions.
d) No food codes for fresh pasta or precooked macaroni are in the 1994-96, 98 CSFII; estimates are based on consum macaroni.

Note: Unless indicated otherwise, all food categories include both regular and lite versions of all food products.

| Table C. Estimated Daily Intake of Inufib™ and Inulin from Proposed Uses by the U.S. Population Ages 2 Years and Older | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Users | | 2-day Average Inufib™ Intake (g/d) | | 2-Day Average Inulin Intake from Dry Inufib™ (g/d) | | 2-Day Average Inulin Intake from Liquid Inufib™ (g/d) | |
| Food Category | N | % | Mean | 90th Percentile | Mean | 90th Percentile | Mean | |
| ALL CATEGORIES COMBINED | 18033 | 99.8 | 9.6 | 19.1 | 8.4 | 16.8 | | |
| Baby foods | 0 | – | – | – | – | – | | |
| Baked goods, lite cakes | 250 | 1.6 | 1.9 | 3.2 | 1.7 | 2.8 | | |
| Baked goods, lite cookies | 365 | 2.3 | 2.0 | 3.8 | 1.8 | 3.3 | | |
| Bars | 748 | 4.0 | 2.7 | 4.6 | 2.4 | 4.0 | | |
| Beverages, fermented milks | 98 | 0.5 | 3.9 | 7.3 | 3.4 | 6.4 | | |
| Beverages, functional | 227 | 1.7 | 13.8 | 25.8 | 12.1 | 22.7 | 11.0 | |
| Beverages, juices and juice drinks | 9146 | 39.9 | 4.7 | 9.4 | 4.1 | 8.3 | | |
| Beverages, milk-based | 2140 | 9.3 | 1.8 | 3.2 | 1.6 | 2.8 | | |
| Biscuits, reduced fat | 6 | < 0.05 | 1.5 | 2.5 | 1.3 | 2.2 | | |
| Breads, conventional | 16565 | 91.9 | 0.3 | 0.6 | 0.3 | 0.5 | | |
| Breads, specialty | 578 | 3.6 | 2.2 | 4.2 | 1.9 | 3.7 | | |
| Candy, hard dietetic | 14 | 0.1 | 0.4 | 0.7 | 0.4 | 0.6 | | |
| Candy, soft dietetic | 5 | < 0.05 | 0.4 | 1.3 | 0.4 | 1.1 | | |
| Condiments | 7153 | 39 | 0.5 | 1.2 | 0.4 | 1.1 | | |
| Cream cheese, reduced fat | 265 | 1.8 | 0.6 | 1.4 | 0.5 | 1.2 | | |
| French fry coatings | 4721 | 25.5 | 0.9 | 1.5 | 0.8 | 1.3 | | |
| Frozen dairy desserts, lite | 1294 | 7.1 | 7.6 | 14.9 | 6.7 | 13.1 | | |
| Icings/glazes, lite | 81 | 0.5 | 0.5 | 0.9 | 0.4 | 0.8 | | |
| Jams and jellies, lite | 141 | 0.7 | 0.2 | 0.4 | 0.2 | 0.4 | | |
| Meat products | 0 | – | – | – | – | – | | |
| Mousse, reduced fat[c] | 13 | 0.1 | 1.3 | 1.9 | 1.1 | 1.7 | | |

## Table C. (cont.) Estimated Daily Intake of Inufib™ and Inulin from Proposed Uses by the Population Ages 2 Years and Older

| | Users | | 2-day Average Inufib™ Intake (g/d) | | 2-Day Average Inulin Intake from Dry Inufib™ (g/d) | | 2-Day Average Inulin Intake from Liquid Inufib™ (g/d) | |
|---|---|---|---|---|---|---|---|---|
| **Food Category** | **N** | **%** | **Mean** | **90th Percentile** | **Mean** | **90th Percentile** | **Mean** | |
| Pancake syrup, lite | 438 | 2.0 | 0.5 | 1.1 | 0.4 | 1.0 | | |
| Pasta fillings | 422 | 2.0 | 1.8 | 3.8 | 1.6 | 3.3 | | |
| Pasta, fresh[d] | 3375 | 18.6 | 2.0 | 4.2 | 1.8 | 3.7 | | |
| Pasta, precooked macaroni[d] | 2133 | 10.9 | 3.0 | 6.0 | 2.6 | 5.3 | | |
| Pizza crust | 3572 | 20.0 | 2.1 | 4.4 | 1.8 | 3.9 | | |
| Potatoes, mashed | 2517 | 13.6 | 2.9 | 6.3 | 2.6 | 5.5 | | |
| Pretzels, soft | 155 | 0.9 | 3.8 | 7.2 | 3.3 | 6.3 | | |
| Processed cheese, reduced fat | 340 | 2.2 | 0.8 | 1.6 | 0.7 | 1.4 | | |
| Pudding mix | 549 | 2.7 | 0.8 | 1.5 | 0.7 | 1.3 | | |
| RTE breakfast cereals | 9049 | 40.8 | 5.5 | 10.1 | 4.8 | 8.9 | | |
| Salad dressings, lite | 1827 | 12.2 | 0.8 | 1.7 | 0.7 | 1.5 | | |
| Sauces and gravies | 13266 | 73.9 | 1.5 | 3.3 | 1.3 | 2.9 | | |
| Snack chips, reduced fat | 350 | 2.0 | 0.5 | 0.9 | 0.4 | 0.8 | | |
| Snack crackers | 2550 | 11.7 | 0.8 | 1.6 | 0.7 | 1.4 | | |
| Soups, dry | 1199 | 5.9 | 0.6 | 1.1 | 0.5 | 1.0 | | |
| Spreads, reduced fat | 4569 | 24.1 | 0.7 | 1.5 | 0.6 | 1.3 | | |
| Surimi | 72 | 0.6 | 0.7 | 1.7 | 0.6 | 1.5 | | |
| Toppings, dessert | 301 | 1.8 | 0.5 | 1.1 | 0.4 | 1.0 | | |
| Tortillas, reduced fat[c] | 2405 | 13.3 | 1.3 | 2.6 | 1.1 | 2.3 | | |
| Vegetarian patties/crumbles | 329 | 2.1 | 0.2 | 0.7 | 0.2 | 0.6 | | |
| Pancake syrup, lite | 438 | 2.0 | 0.5 | 1.1 | 0.4 | 1.0 | | |
| Pasta fillings | 422 | 2.0 | 1.8 | 3.8 | 1.6 | 3.3 | | |

## Table C. (cont.) Estimated Daily Intake of Inufib™ and Inulin from Proposed Uses by the Population Ages 2 Years and Older

| | Users | | 2-day Average Inufib™ Intake (g/d) | | 2-Day Average Inulin Intake from Dry Inufib™ (g/d) | | 2-Day Average Inulin Intake from Liquid Inufib™ (g/d) | |
|---|---|---|---|---|---|---|---|---|
| **Food Category** | **N** | **%** | **Mean** | **90th Percentile** | **Mean** | **90th Percentile** | **Mean** | |
| Whipped toppings, lite | 101 | 0.7 | 0.6 | 1.7 | 0.5 | 1.5 | | |
| Yogurt, reduced fat | 1191 | 6 | 3.4 | 6.4 | 3.0 | 5.6 | | |

Data source: USDA Continuing Survey of Food Intakes by Individuals (CSFII), 1994-96, 1998. Breastfeeding infants and children w from the analysis. Estimates are based on food consumption reported by individuals who provided two 24-hour diet recalls and ma use levels consistent with maximum Frutifit use levels specified in FDA GRN # 118.

a) Dry Inufib™ is 88% inulin by weight.
b) Liquid Inufib™ is 80% inulin by weight.
c) No food codes for reduced fat forms of this food category are in the 1994-96, 98 CSFII; estimates are based on consum versions.
d) No food codes for fresh pasta or precooked macaroni are in the 1994-96, 98 CSFII; estimates are based on consum macaroni.

Note: Unless indicated otherwise, all food categories include both regular and lite versions of all food products.

**10.18** Attachment 18

Pages 000169-000231 have been removed in accordance with copyright laws. The list of the removed references can be found on pages 000004-000005.

#582
GRN000582

NSF International

June 16, 2015

VIA email
Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-255)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Attention: Mr. Richard Bonnette; cc. Dr. Paulette Gaynor

RE: GRAS Notification – Premium Agave Inulin – Exemption Claim

Dear Mr. Bonnette:

On behalf of IIDEA of Tlaquepaque Jalisco, México, and as their agent, we are providing this signed and dated GRAS exemption claim for the subject GRAS Notification, in accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized as Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)]. We respectfully request that this document be appended to our May 20, 2015 submission once it is filed as a notice.

As the notifier, IIDEA (Industrializadora Integral del Agave SA de CV; Av. Periférico Sur 7750, Tlaquepaque Jalisco, México, FDA registration number: 13439186334) has determined that its premium agave inulin product, trade name "Inufib™" is Generally Recognized As Safe (GRAS) in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. Inufib™ is the trade name used by IIDEA, for the inulin-type fructans derived from the piñas (stems, also known as cores, hearts, or pines) of the agave plant, *Agave tequilana* Weber var. *azul*, commonly known as "blue agave" and "weber's blue agave." Inufib™ is intended for use as an ingredient in a variety of foods and beverages in which it serves as a bulking agent or a source of reduced energy carbohydrate, for uses as a sugar replacer, fat-replacer and/or texture modifier, and at levels typically ranging from 2 – 8 g inulin per serving. This GRAS determination was made through scientific procedures, pursuant to 21 CFR § 170.36, and in concert with a convened panel of experts qualified by scientific training and experience. Accordingly, agave inulin, meeting the specifications as described in the subject notification, and used according to the conditions described in the subject notification, is exempt from pre-market approval requirements for food ingredients.

Should you have any questions or require any additional information regarding the subject GRAS Notification or exemption claim please to not hesitate to contact me by email at: jenglish@nsf.org.

Sincerely,
(b) (6)

J. Caroline English, Ph.D., DABT
Senior Principal Toxicologist
NSF International
jenglish@nsf.org

SUBMISSION END